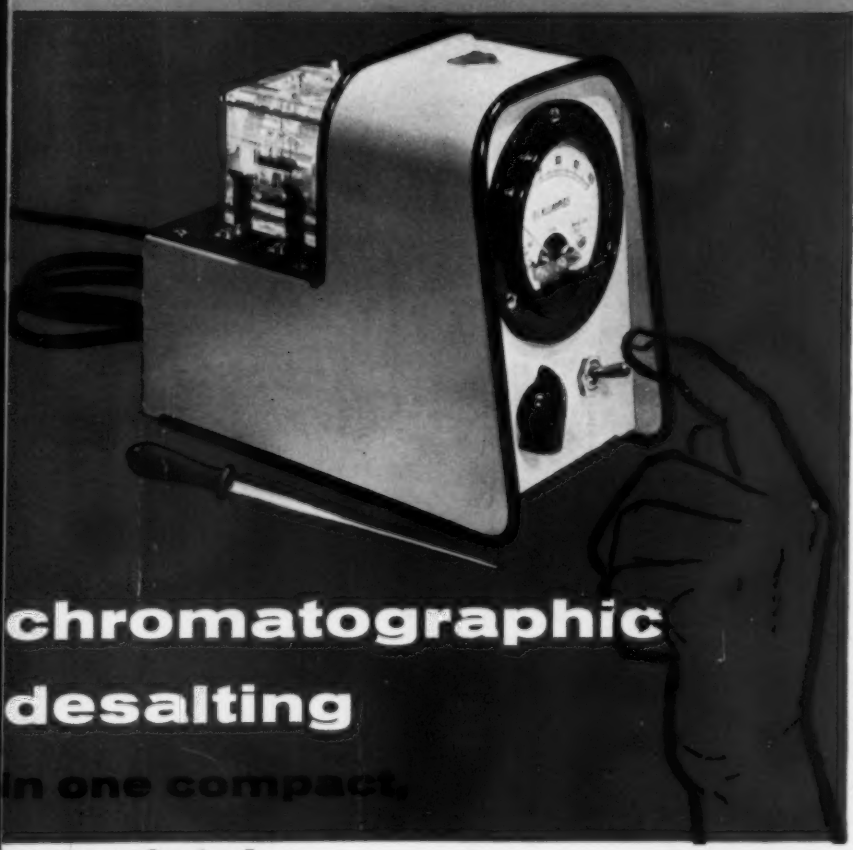
LABORATORY PRACTICE

RESEARCH · CONTROL · TEACHING

VOL. 10 No. 9 Published Monthly SEPTEMBER 1961



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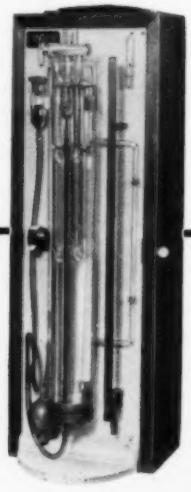

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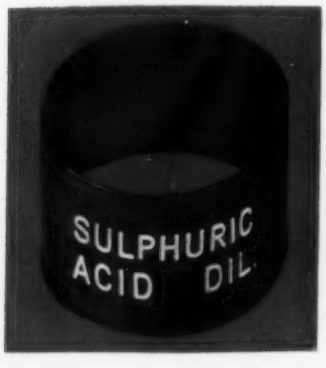

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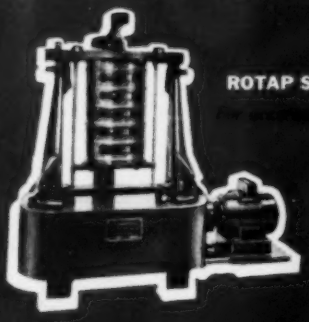

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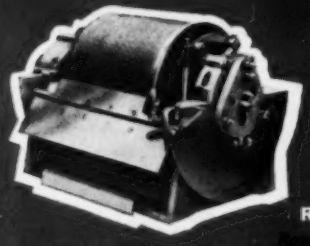

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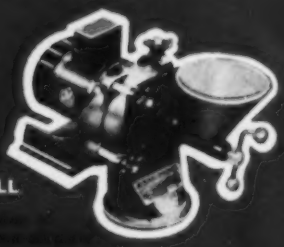

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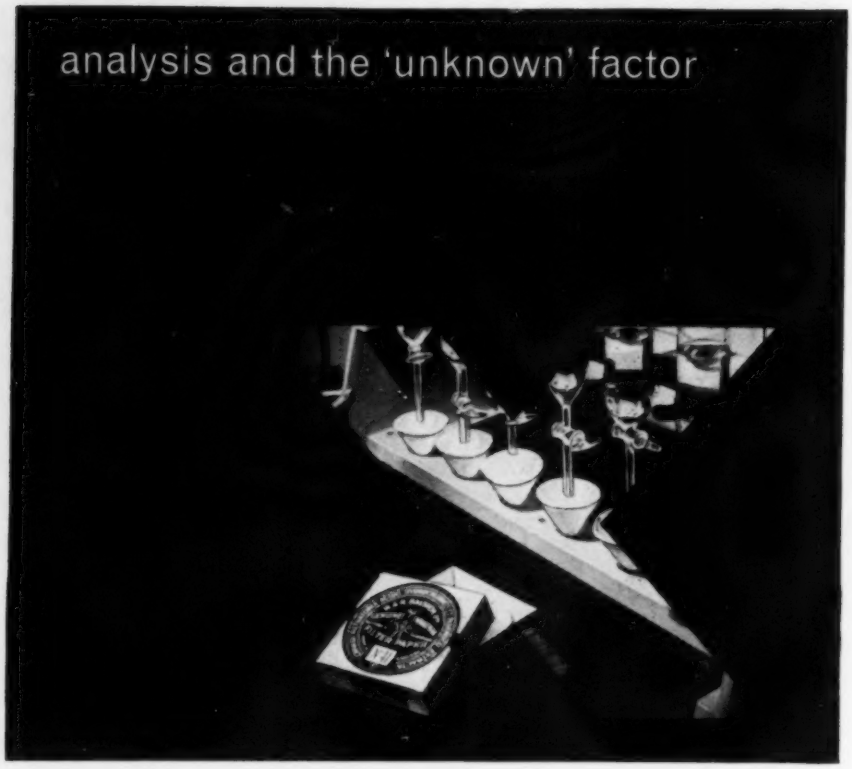
analysis and the 'unknown' factor



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The above quotation is an extract from the 'Filtration Section' of the Whatman catalogue. Other Whatman literature available upon request includes a 'Buyers' Guide' and technical leaflets concerning papers for Chromatography, Ion Exchange, Air Filtration and Special Fibre Papers.

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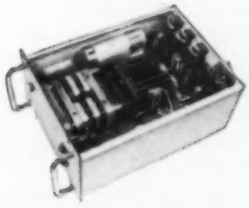

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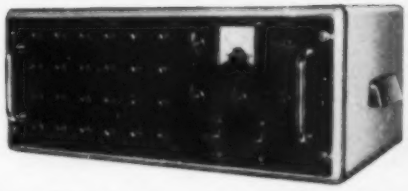

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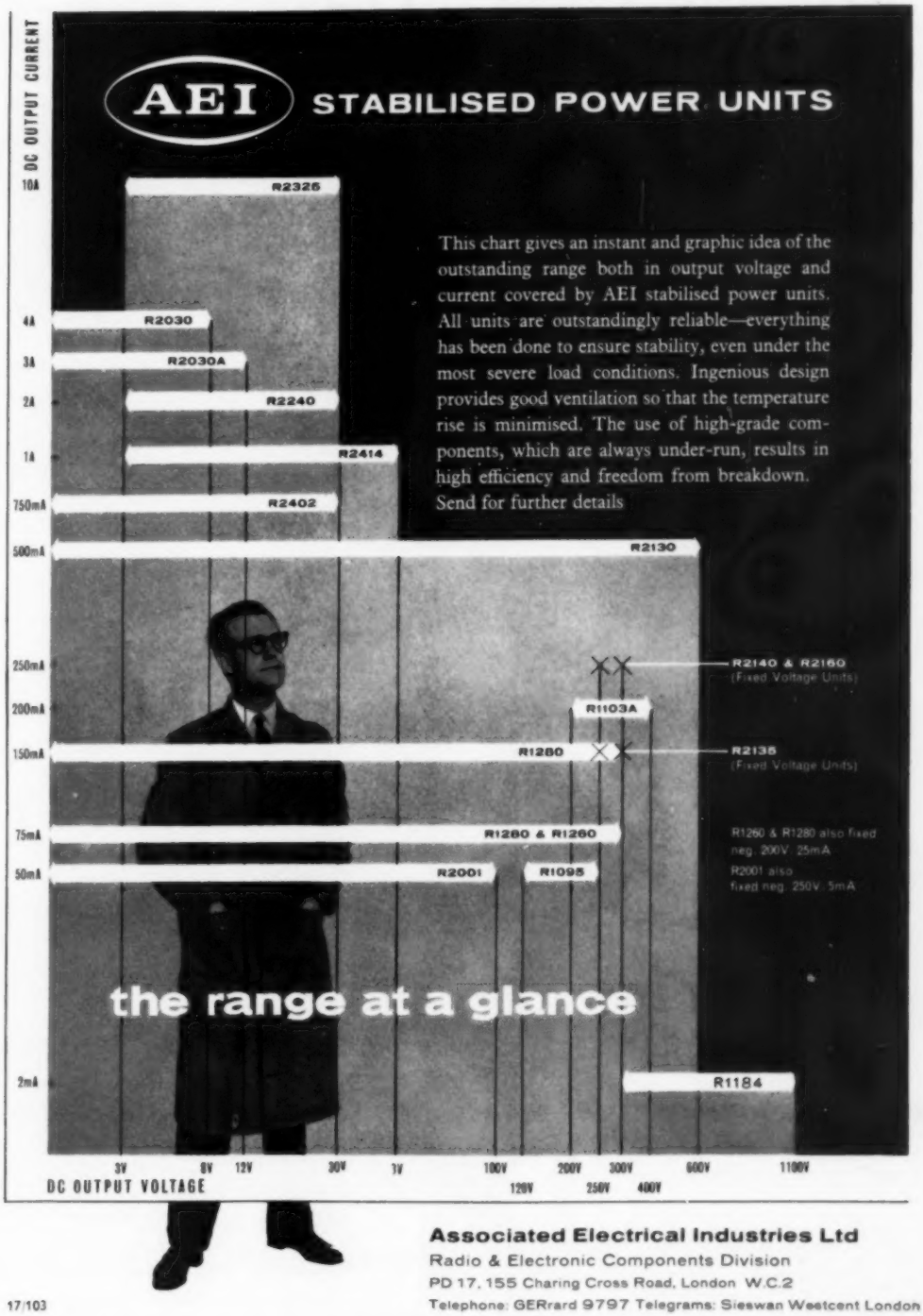

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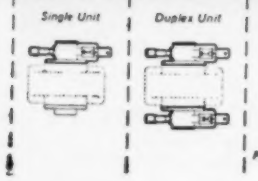

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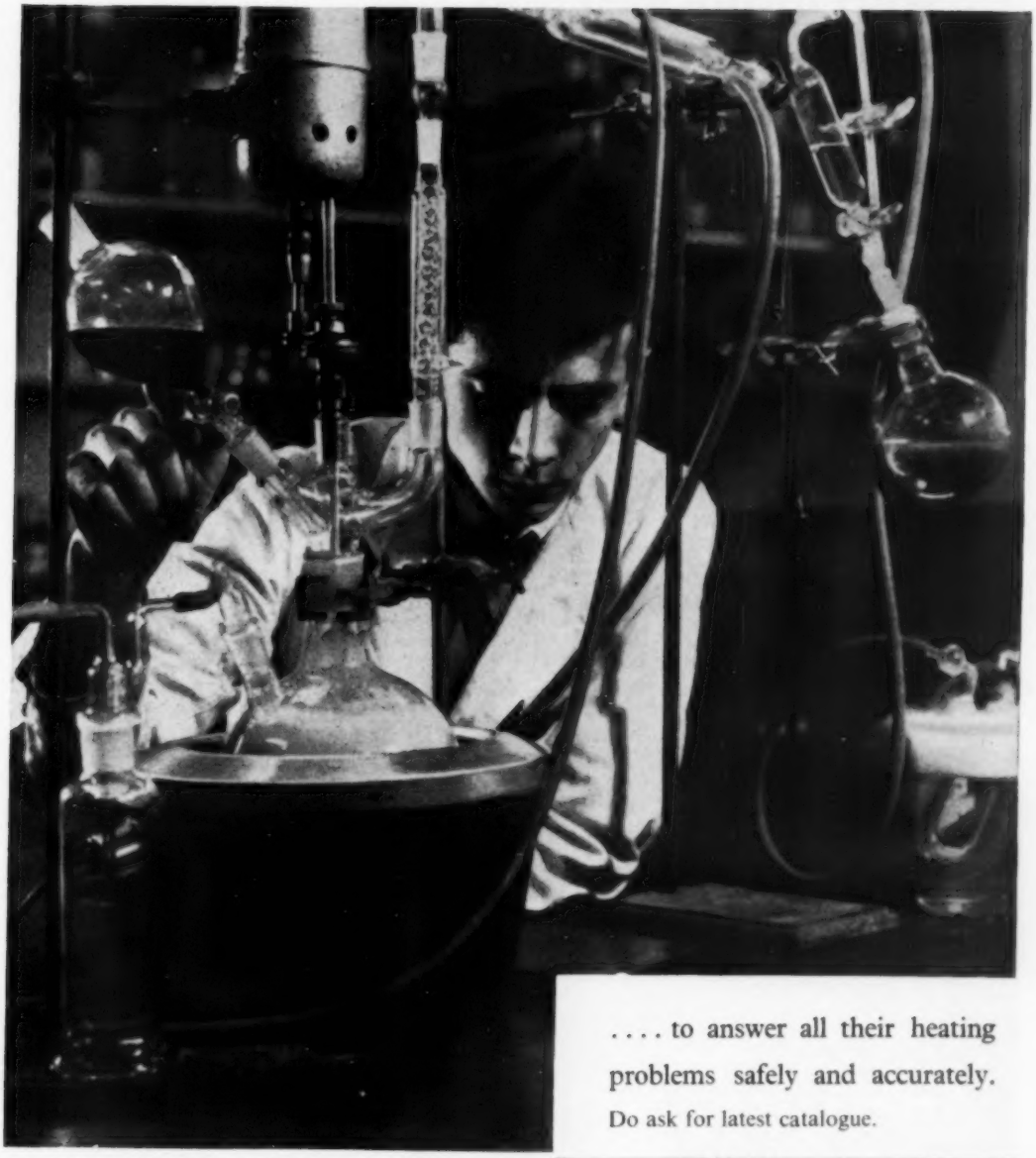

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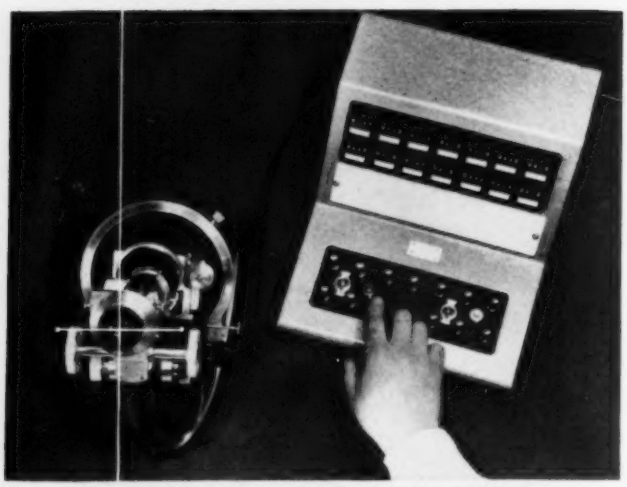

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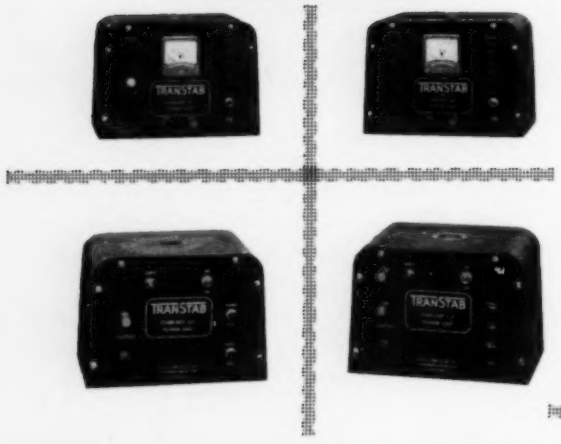

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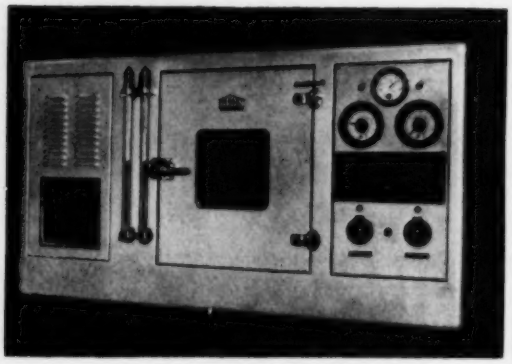

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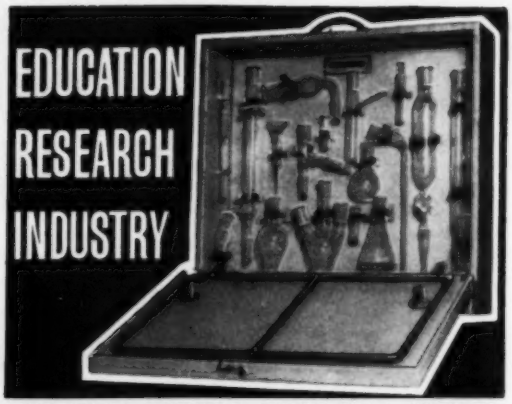

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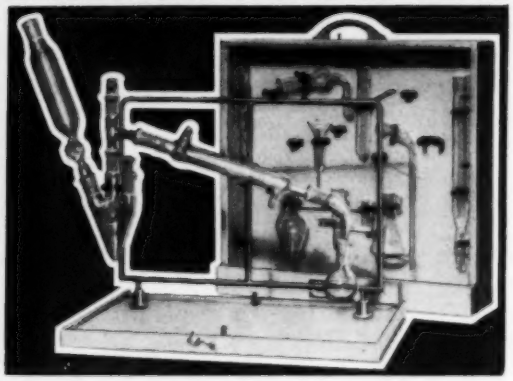

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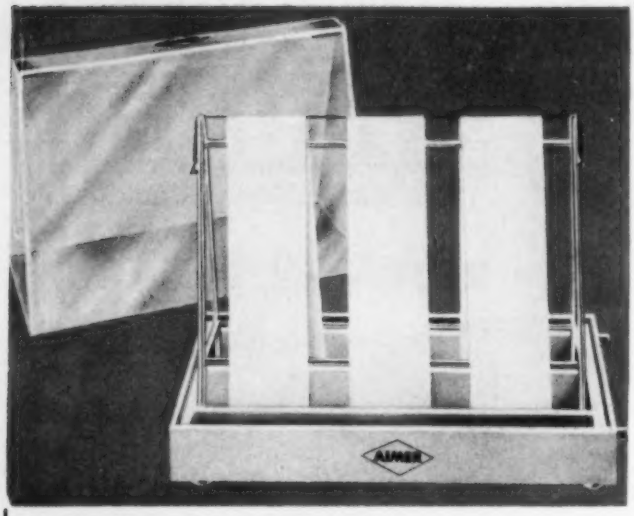

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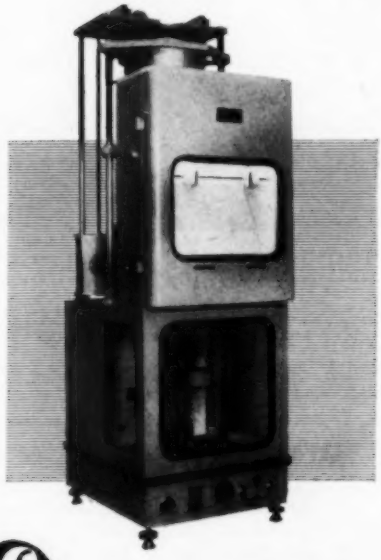

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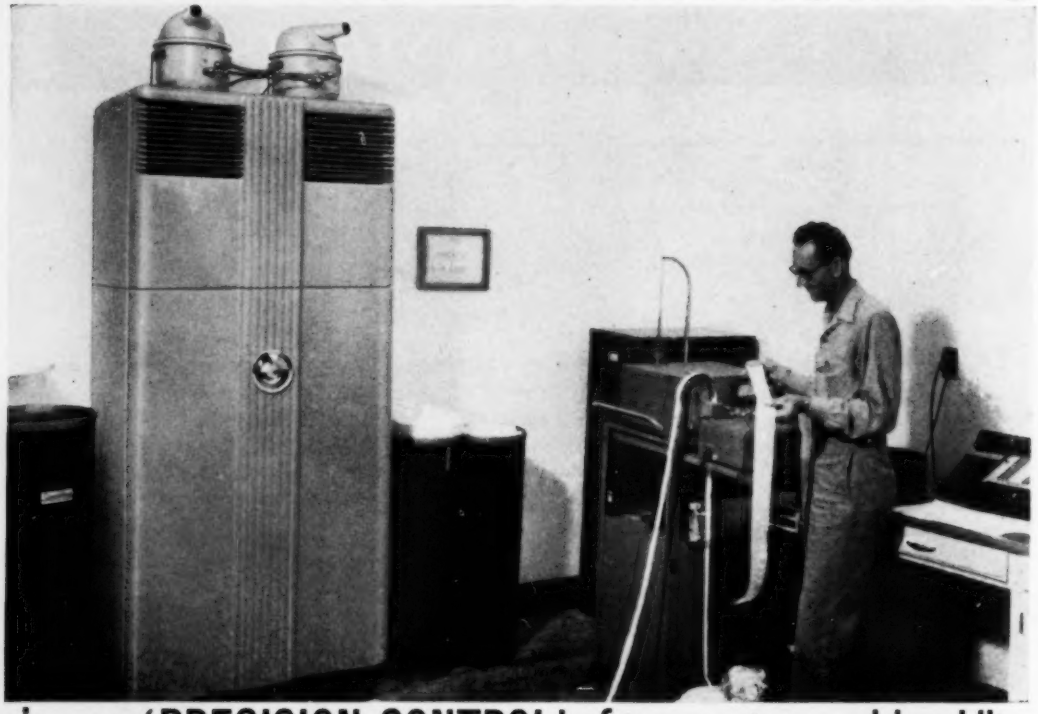

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EDITORIAL

Introductions THE number of papers which have been published on even the most abstruse branch of science is now so large that the scientist setting out to write his paper is confronted with a difficult problem at the start. Any serious attempt to survey the subject comprehensively and do justice to previous workers in the field leads to the compilation of a monograph. Today editors severely limit the length of scientific papers and require authors to reduce their historical introduction to a bare minimum. This poses a difficult problem for the author for he should not only briefly summarize the present state of knowledge on the subject of his paper, but he should also clearly indicate the contribution of previous workers, for he is in a sense standing on their backs.

It is usual to see a few of the classical references quoted in the introduction and these seem to be copied from paper to paper. The author, or if he is a junior his professor, is usually familiar with the most recent work (often it is a recent paper which has stimulated the work) and so reference is made to two or three very recent papers and often to the 'personal communication'. The result is that the intermediate work, say from 1920 to 1950, may be completely ignored. It is of course a considerable task to muster all the original work on any subject since its beginning and very few workers do this, relying on books, monographs and reviews. The danger of this procedure is that the authors of such reviews and monographs may easily give a one-sided view of the subject, naturally giving most information about their own contributions. It follows that if the literature is not studied as it should be the author writes in ignorance of much previous work and may even report 'discoveries' which were described some 20 or 30 years ago. No doubt it is only the rare unscrupulous scientist who deliberately passes off other people's ideas and discoveries as his own, but some papers give the impression that the author is either deliberately ignoring or is ignorant of previously reported work. This is especially true of work in journals foreign to the author. Thus American workers may not be familiar with British journals, British workers with German journals, and so on.

In our younger days it used to be said half seriously that the research worker embarking on a new investigation should either read the whole of the literature on the subject, or none of it. There is a considerable germ of truth in this idea. The former leads to a well-informed mind, and the latter method leaves the mind unbiassed and free to develop its own ideas. It is clearly the responsibility of the professor or other supervisor to guide the young scientist on this matter, and he presumably should be completely familiar with the subject. Instruction in the methods of searching the literature should be a most important part of training in research methods. There is no more important research apparatus than the library and this should be brought home to the scientist at the start of his career.

It has become fashionable for the confident young scientist to write 'Very little information appears to be available on . . . ' and this may be rather startling and even pathetic when the experienced worker knows that many hundreds of papers and a few monographs and reviews have appeared. As he gets older, or if he has a shrewd professor, the wording becomes more cautious, for example 'Very little reliable information . . .' or 'Very little accurate information . . . ', a delightfully ingenuous and at the same time devastating way of dealing with one's predecessors.

Failure to quote previous work adequately is a sin to which we must all plead guilty, but the unforgivable sin is to quote only those papers which support the idea to which the author is leading. It is easy enough to prove anything by selection of data; the wise author, if anything, emphasizes the evidence against his theories and leaves it to the reader to draw his own conclusions.

Royal Society and Nuffield Foundation Awards

The following is the remainder of the recent awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme.

Dr. J. N. Phillips, of the C.S.I.R.O. division of plant industry, Canberra, to enable him to extend his physico-chemical studies on plant and animal pigments at Imperial College, London, for about eleven months from December 1961.

Dr. Muriel L. Tomlinson, university demonstrator in organic chemistry, Oxford, to enable her to study polydentate compounds at Sydney University for about three months early in 1962.

Prof. D. Walker, professor of physics, Victoria University of Wellington, to enable him to make a study of experimental research work currently of interest to workers with low energy Van de Graaff accelerators (or similar machines), and to study the working of the National Institute for Nuclear Science in Britain, for eight months from June 1962.

Dr. H. B. S. Womersley, reader in botany, University of Adelaide, to assist him with maintenance costs at Vancouver for three months from April 1962 while making ecological comparisons of the intertidal biology (especially marine algae) of southern Australia and the western North American coast.

Society for Applied Bacteriology

The Annual Meeting of the Society for Applied Bacteriology was held in Wye on July 11. The following are now officers of the Society: *Hon. President*, Mr. G. Sykes; *Hon. Secretary*, Dr. Ella M. Barnes; *Hon. Treasurer*, Mr. G. Elis Jones; *Hon. Editors*, Dr. S. E. Jacobs and Dr. D. J. Jayne-Williams; *Hon. Publications Manager*, Mr. A. H. Walters; *Hon. Advertising Manager*, Mr. E. J. Mann. *Members of Committee*, Mr. B. M. Gibbs, Dr. N. D. Harris, Dr. Agnes A. Nichols, Dr. J. R. Norris, Dr. A. M. Paton, Dr. Patricia M. Scholes, Dr. A. Seaman, Dr. Joan Taylor, Dr. G. C. Ware.

GAS CHROMATOGRAPHY—RECENT DEVELOPMENTS AND APPLICATIONS

by R. P. W. Scott, D.Sc., F.R.I.C.

Technical Department, Benzole Producers Ltd., Watford

The effect of film thickness and operating temperature on the performance of capillary columns is described and examples of automatic monitoring of gas streams are given. The application of gas chromatography to examination of products from a coke oven and the use of the technique in the study of reaction kinetics and trace analysis is considered. The limitations of the partition system of high-temperature chromatography are discussed and a description given of recent work utilizing adsorbents for high-temperature separations.

Introduction

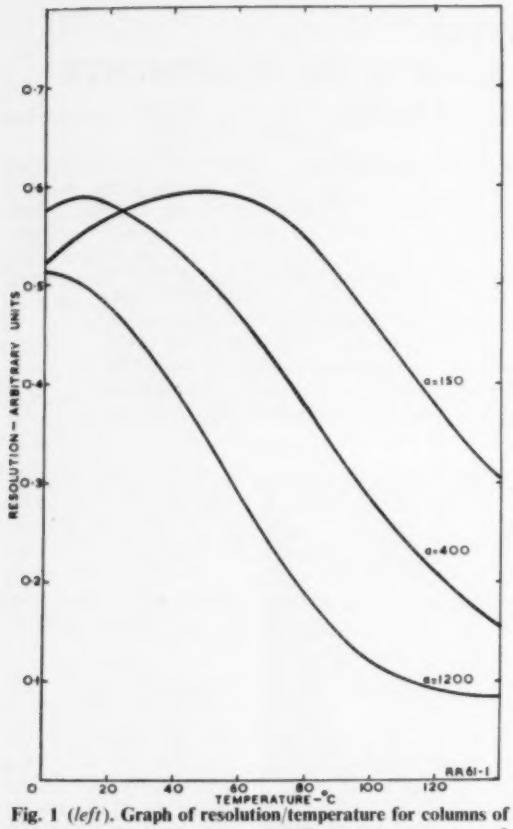
Probably the most important developments in gas chromatography in recent years have been the high sensitivity detectors of Lovelock[1] and MacWilliam and Dewar[2] and the capillary columns of Golay[3]. With the advent of the Argon detector[1] and the flame-ionization detector[2] the separation of even multicomponent mixtures could be carried out with a few micrograms of sample. This allowed the development of the high-efficiency conventional columns[4] and the capillary column of Golay[3] neither of which could be operated satisfactorily with the less sensitive type of detectors as the charge required for analysis would heavily overload either type of column.

R. P. W. Scott, D.Sc., F.R.I.C.

High efficiency columns have widened still further the field of applications of the technique and due to the low pressure drop across the capillary column and its inherent low-plate capacity this type of column has been developed to give extremely short analysis times[5,6]. Columns of over a million theoretical plates are now available or, alternatively, analysis times can be reduced to a few seconds. Developments in gas chromatography during the last year since the lecture by Dr. A. T. James have been confined largely to improvements in resolution, reduction of analysis times, improved instrumentation and the application of the technique to even more problems of industry and research. The scope of this lecture only allows a limited number of examples to be given, but these have been chosen to demonstrate the various aspects of the techniques that have recently aroused interest.

Capillary Columns

The main advantages of the capillary column over the conventional column are the absence of the multipath effect and the low pressure drop across the column. The multipath effect in the conventional column is due to the different path lengths the carrier gas has to travel when winding its way between the particles of the support, resulting in 'band spreading' and a fall in efficiency. The high pressure drop across a conventional column, necessitating a high absolute pressure within the column, reduces the diffusivity of the solute in the gas phase. This, in turn, reduces the efficiency of the column, as a low value for the height of the theoretical plate is dependent on fast diffusion rates. Thus, due to the effect of pressure a practical limit is set for the length of a conventional column at about 100 ft., giving an efficiency of about 50,000-100,000 plates. Conversely capillary columns of several thousand feet in length can be employed giving efficiencies of several million plates. Ultimately, the same restrictions due to increase in column pressure will apply to a capillary column, but at a much higher level of efficiency.



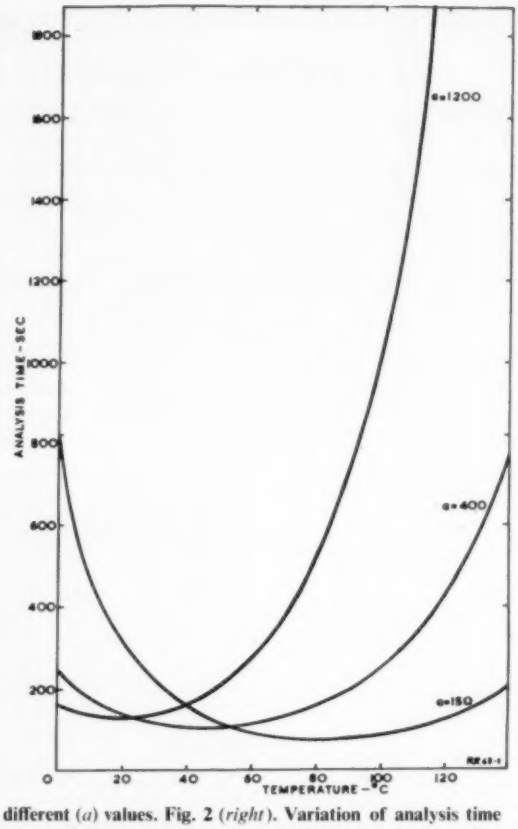


Fig. 1 (*left*). **Graph of resolution/temperature for columns of different** (*a*) **values. Fig. 2** (*right*). **Variation of analysis time with temperature for columns of different** (*a*) **values. (a** $= \frac{r}{2df}$ **where r is the radius of a column having a film thickness df).**

It should be emphasized, however, that high efficiencies from a capillary do not necessarily result in a high resolving power. The rate of diffusion of a pair of solutes in the stationary phase on a given column can be increased by reducing the film thickness. This will produce sharper peaks and higher efficiencies. However, the separation between the peaks is a function of the total quantity of stationary phase on the column and this has also been reduced in decreasing the film thickness. This means that the peaks have become sharper, but also closer together so that although the column has an increased efficiency, it may have a poorer resolving power. At one extreme where there is no stationary phase on the column the peaks will be extremely sharp but superimposed on one another; at the other extreme with a very thick film the peak maxima will be far apart but the peaks themselves will merge together due to band spreading resulting from slow diffusions in the thick film of liquid phase. There is, in fact, an optimum film thickness that will give the maximum resolution between two given substances on a given column.

The rate of diffusion of the solute in the gas and stationary phases can also be increased by raising the operating temperature with a resulting increase in column efficiency. Raising the operating temperature however, also brings the peaks closer together so that an increased column efficiency does not necessarily result in improved resolution. For two given solutes on a given column at high operating temperatures, the peaks will be very sharp, but also very close and poorly resolved. Conversely, at low operating temperatures, the two solutes may still be poorly resolved as the peaks may be far apart but very broad due to slow rates of diffusion. It is obvious that for the separation of a particular pair of solutes on a given column there will be an optimum operating temperature as well as an optimum film thickness that will provide maximum resolution. There will be similar optimum conditions for minimum analysis time. An example of the effect of temperature on resolution and analysis time is given in Figs. 1 and 2.

Automatic Monitoring by Gas Chromatography

The high resolving powers and short analysis times that can be readily obtained from capillary columns makes

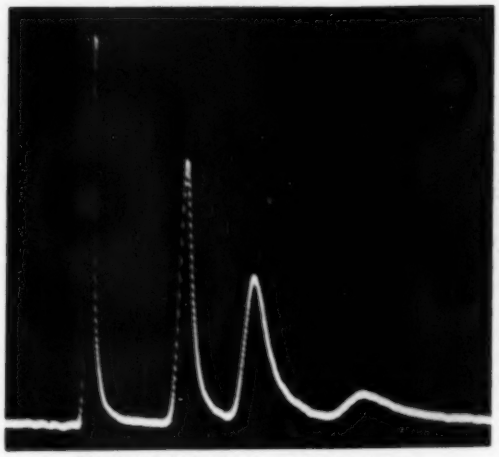


Fig. 3. Analysis of an anaesthetic mixture.

them an attractive system for use in plant monitoring. Such a system however, requires a representative and reproducible sample of about 1 μg to be placed on to the column by an automatic injection device. In practice it is necessary to take a considerably larger sample through a by-pass system on to the capillary column and perhaps reject 98 per cent of the sample to waste. It is perhaps obvious that the first application of this kind was for the analysis of volatile solutes already existing in a gas due to the relative simplicity of injection of a sample in this form. It is then only necessary to place on to the column via a by-pass system a reproducible and representative gas sample possibly using a method which has already been employed with conventional columns. Such systems have been used for the analysis of the paraffinic and aromatic hydrocarbons contained in coal gas and for the analysis of the anaesthetic content of of a patient's breath while under anaesthesia. An example of the latter analysis is shown in Fig. 3. The latter analysis of an anaesthetic mixture is displayed on an oscilloscope. A simplified form of the apparatus used for monitoring coal gas is shown as a block diagram in Fig. 4. Sample and carrier gas flow through the sample device in separate streams. At predetermined intervals, signals from the timer of oscilloscope actuate the injection device and place a known volume of sample in line with the carrier gas stream. The sample is then swept through the by-pass system and passed on to the column. At the end of the column is situated the detector, amplifier and recorder or oscilloscope. The automatic sampling of liquids for a capillary column is considerably more difficult. It is necessary to take a representative and reproducible sample of 1 μg. from a liquid stream, vaporize it without loss of any of the components due to adsorption on surfaces with which it is in contact, and place it on the column. This operation has also to be achieved with a minimum of time lag between injection device and the sample point. Many approaches to this problem are being investigated, one method that appears to work satisfactorily is shown diagrammatically in Fig. 5. The liquid sample from a still or fractionating column is pumped through an atomizer where the sample is vaporized in a jet of nitrogen. The container in which the sample is atomized carries a heater to ensure

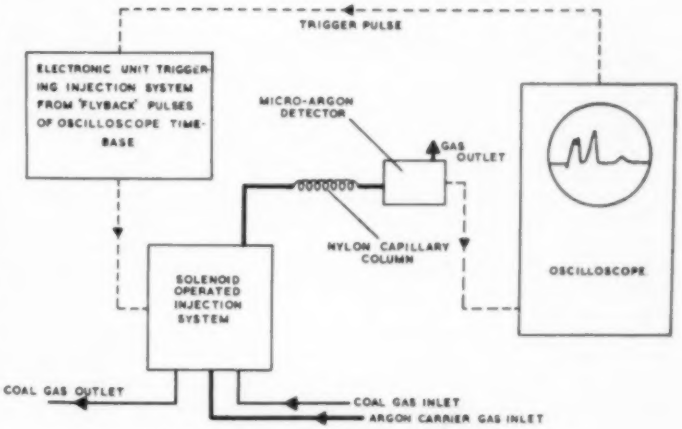


Fig. 4. Block diagram of coal-gas monitor.

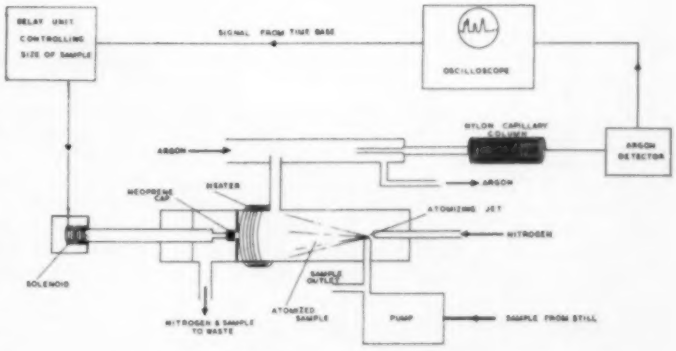


Fig. 5. Block diagram of sampling system.

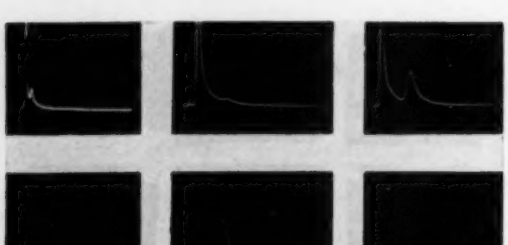


Fig. 6. Stages in the fractionation of a hydrocarbon mixture.

complete vaporization and in the periods between injections the sample in the nitrogen stream passes to waste. At the end of each scan of the oscilloscope a solenoid is triggered by the fly-back signal and the exit of the sample to waste sealed with a Neoprene cap. A pressure is then momentarily built up in the vaporizer unit resulting in a sample of the vapour being forced into the argon stream flowing through the sample divider onto the capillary column. The quantity of charge placed on the column is dependent on the period over which the exit port from the vaporizer unit is sealed off. This period is controlled by the time delay unit that actuates the solenoid from the pulse derived from the oscilloscope. By careful adjustment this sample system can be made representative and reproducible, and the analysis of the products from a fractionating column can be monitored every minute or less. Fig. 6 shows a series of chromatograms obtained on an oscilloscope during the laboratory fractionation of a hydrocarbon mixture. A 10 ft. length of 0·020 in. bore nylon tubing coated with dinonyl phthalate was used as the partition column in conjunction with a micro-argon detector.

Plant Research

Gas chromatography can also be an invaluable aid to plant research. Plant conditions may be varied and the effect of these variations on the quality of the product easily studied. Among many other applications of this type the changes in the composition of benzole contained in the coal gas produced during the carbonizing period of a coke oven have been monitored. Samples of gas were taken, prior to the benzole scrubbing tower, every thirty minutes during the eighteen-hour coking period. These samples were injected on to a 40 ft. conventional column operating at an inlet pressure of 150 p.s.i. This was achieved by means of a special high pressure gas injection device. The column functioned at an efficiency of 15,000 theoretical plates and was used in conjunction with a micro-argon detector, an amplifier and a potentiometric recorder. A pair of chromatograms showing the analysis of two samples of gas taken after two and thirteen hours carbonization respectively is shown in Fig. 7 and a graph showing the changes in concentration of several components of the gas during the whole carbonizing period is shown in Fig. 8. Wherever the products of a plant are sufficiently volatile to permit analysis by gas chromatographic techniques the characteristics of the plant producing these substances may be examined. Plant research is still a very wide field for application of the technique.

The Study of Reaction Kinetics

The applications of the technique to normal laboratory research are too numerous to be listed. It has been applied to the separation of widely-varying volatile mixtures from essential oils to crystalline waxes and from interhalogen compounds to the volatile metallic halides. Relatively few papers have, however, been published showing the application of the technique to the study of reaction kinetics. One example of the use of gas chromatography for this purpose is an investigation of the thermal decomposition of hydrocarbons. The problem was to investigate the thermal decomposition of aromatic and paraffinic hydrocarbons existing in vapour form in coal gas when in contact with hot coke at various temperatures and at various contact times.

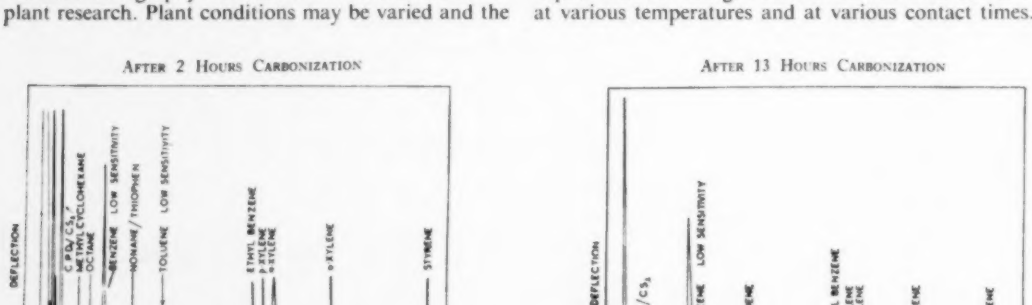


Fig. 7. Chromatograms of benzole in gas.

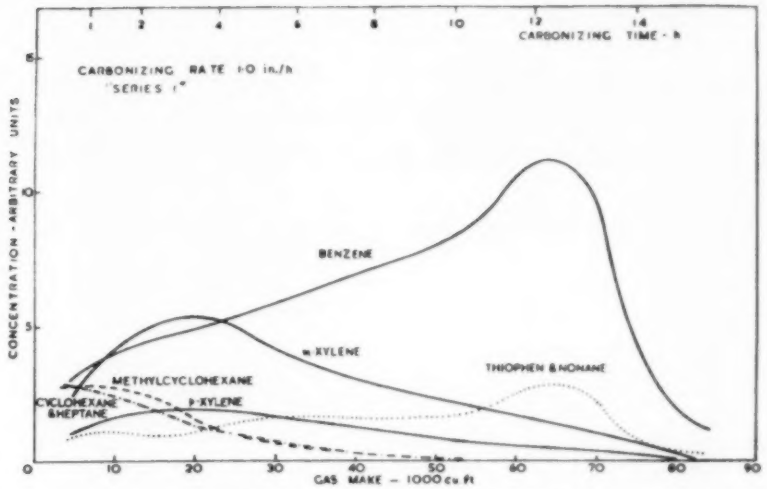


Fig. 8. Variation of concentrations of hydrocarbons in gas during carbonization.

(Note: Concentration units differ for each compound)

The apparatus used is shown in Fig. 9. Coal gas derived from a cylinder is first stripped of its inherent benzole by passage through a furnace at about 1,000°C. The gas then passes to a sample vaporizer where it takes up a predetermined vapour concentration of the hydrocarbon under examination. The gas and vapour then flow over a bed of heated coke maintained at a given temperature by means of a thermostatically-controlled furnace and thence to the injection system of a gas chromatograph. Primarily the pyrolysis furnace is by-passed and a sample of the unpyrolysed gas placed on the column, this gives a reference chromatogram of the original sample concentration. The flow of sample is then diverted through the hot coke and time allowed for equilibrium to be obtained. A sample of the pyrolysis products is then taken and placed on the column. This procedure has all the advantages of the dynamic system of measurement. The initial concentration of the hydrocarbon in the gas may be varied by adjustment of the vaporizer and contact times altered by changing the gas flow-rate through the pyrolysis furnace. The injection system simultaneously places charges on twin columns one containing a polar stationary phase and the other a non-polar stationary phase and this aids in the identification of the pyrolysis fragments obtained. The change in the thermal decomposition of toluene and the simultaneous production of benzene at various pyrolysing temperatures is shown in Fig. 10. Several examples of the use of gas chromatography in the identification of products of pyrolysis have been described in the literature e.g. examination of polymers[7], identification of substances of biological origin[8].

Trace Analysis

Both the flame-ionization detector and the argon detector are capable of detecting vapour concentrations of less than 10^{-10} gm. per ml. which makes any system using them ideal for trace analysis. Chromatographic columns incorporating these detectors and the less sensitive types have been used in the determination of traces of acetylene in oxygen[9], analysis of fruit odours[10], and cigarette smoke[11], mine atmospheres[12,13], and in examination of atmospheric pollution[14,15].

The sampling technique depends to some extent on the concentration of the solutes concerned. Where the sample is a gas and the concentrations are extremely low a concentration technique[16] may be necessary. The gas can be passed through a small length of tubing packed with a support and stationary phase, this acts as an extraction vessel, but care must be taken not to use too great a volume of gas sample as eventually the substances of interest will be eluted from the sample tube. After sampling, the tube is placed in line with a chromatographic column and a high sensitivity detector and a chromatogram of the trace components obtained. This procedure has been applied to the determination of the hydrocarbons present in the atmosphere existing in a coal stack and a chromatogram of such a sample is shown in Fig. 11. The relative amounts of the substances present are given in Table I.

High Temperature Chromatography

Theoretically, providing the components of a substance have a sufficiently high vapour pressure to give a response on one of the high-sensitivity detectors, that substance can be analysed by gas chromatography. However, although many high molecular weight substances can be raised to a temperature at which they are still stable and at the same time provide vapour in sufficient concentration to be detected by the argon or flame-ionization detector, they cannot necessarily be chromatographed at this temperature. This is due to the high volatility of the liquid phase that is employed, or decomposition products produced at high temperature.

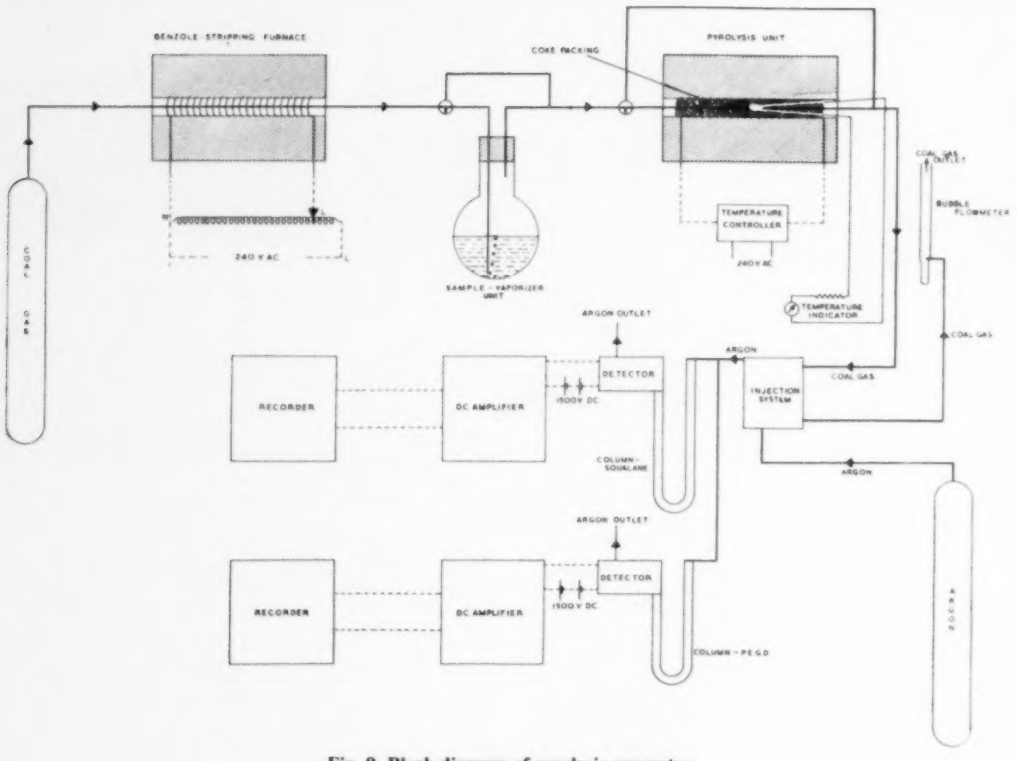


Fig. 9. Block diagram of pyrolysis apparatus.

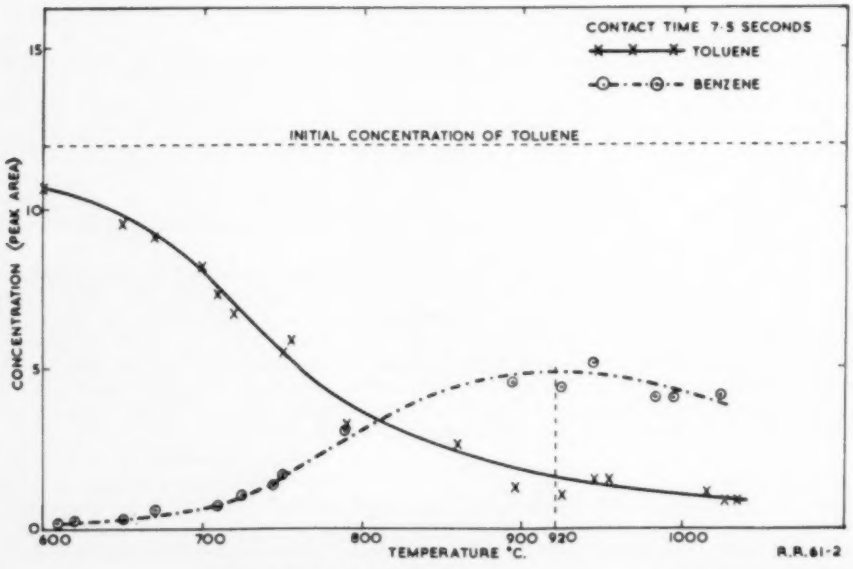


Fig. 10. Graph of pyrolysis temperature against concentration for benzene and toluene.

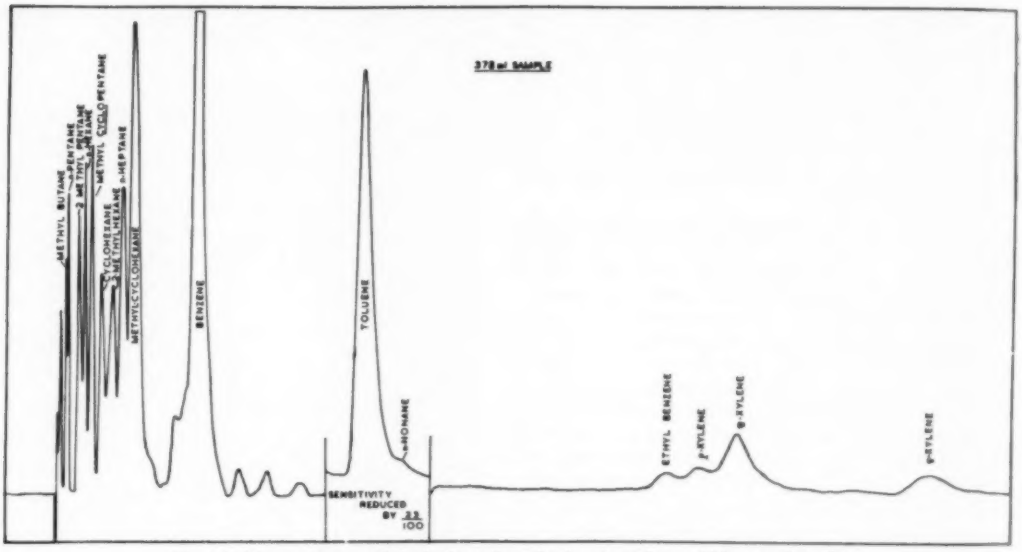


Fig. 11. Chromatogram of the hydrocarbons in the atmosphere within a coal stack.

TABLE I

Hydrocarbons in air sample

Hydrocarbons	*Concentration in micrograms/litre*
iso-pentane	0·2
n-pentane	0·4
2-methylpentane	0·3
n-hexane	0·3
methyl*cyclo*pentane	0·6
*cyclo*hexane	0·5
n-heptane	1
methyl*cyclo*hexane	2
benzene	4
toluene	30
ethylbenzene	0·7
p-xylene	0·5
m-xylene	2
o-xylene	1

Probably the best liquid phase for high temperature work is the Apiezon L. grease. This may be used up to temperatures of about 300°C. Some silicone compounds have been used as stationary phases above this temperature, but these have a decomposition product that affects the stability of high sensitivity detectors.

Another practical limitation to the chromatographic separation of high molecular weight substances is the temperature at which the detector will function. Although in principle, the argon detector should still function at high temperatures, in practice insulation becomes more difficult, particularly at voltages of 1,200 or more. For this reason, the flame-ionization detector appears to be more satisfactory for very high temperature chromatography. However, even if the flame-ionization detector is employed, the limitation due to the volatility of the stationary phase still holds.

An alternative approach to this problem has been made by C. G. Scott[17] who has used de-activated alumina adsorbents instead of a liquid phase. Untreated alumina could not be used in this manner as asymmetric peaks would be formed and so he developed a method of de-activation of the alumina with caustic soda. Using a conventional column a few feet long in conjunction with a flame-ionization detector, he has chromatographed hydrocarbon waxes up to carbon number 45. A chromatogram of such a mixture is shown in Fig. 12.

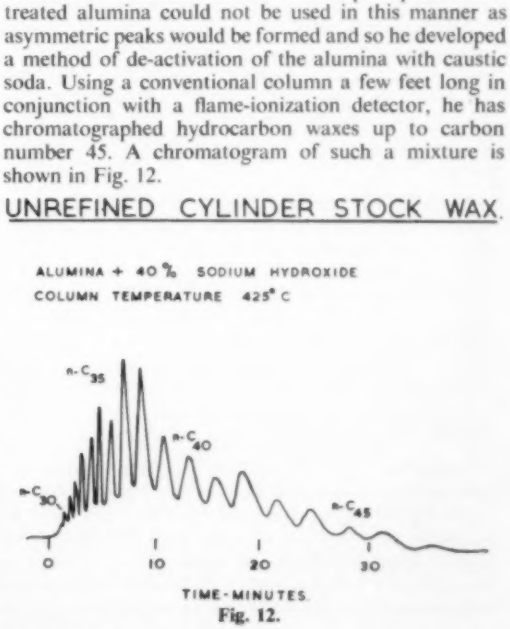


Fig. 12.

The merging of the peaks at the base is due to the presence of isomers. It is interesting to note that the column temperature was 425°C. and the stability of the base line produced by the detector was still very good.

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RADIO-FREQUENCY METHODS IN ANALYTICAL CHEMISTRY

by W. H. Lee, Ph.D., F.R.I.C.

Battersea College of Technology

I SUPPOSE that when most of us were taking courses in chemistry, the only mention of *radio-frequency* was in connection with the classical work of Falkenhagen, on the relaxation-times of the ionic atmosphere in a solution. Today, however, most laboratories make use of radio-frequencies, both in research, and in qualitative and quantitative analysis. I want to consider what advantages the use of this particular region of the electromagnetic spectrum has to offer. For this purpose, I shall define 'radio-frequencies' as electromagnetic fields oscillating at frequencies between about one million cycles per second (one megacycle per second, Mc/s.) and 50 Mc/s., that is, within the medium and short-wave radio transmission bands. We may divide our discussion into:

(*a*) determinations which can be more conveniently made at radio-frequency than at some other frequency, e.g. conductivity measurements without internal electrodes, and dielectric constant measurements;

(*b*) determinations which can *only* be made because of the availability of generators of this range of frequencies, e.g. measurement of dipole moments, applications of nuclear magnetic resonance, and of the acoustic interferometer. The usefulness of electromagnetic radiation does not end at 50 Mc/s. At much higher frequencies we can measure electron-spin resonance and study the rotations of molecules; however the techniques involved in working at several thousands of megacycles per second are very different from those of our present enquiry.

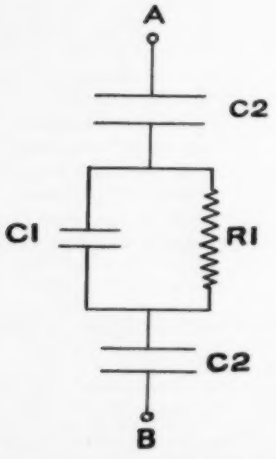


Fig. 1. Equivalent electrical circuit of high frequency conductivity cell.

W. H. Lee, Ph.D., F.R.I.C.

High Frequency Conductivity

It is well known that very accurate and precise conductivity measurements may be made at audio-frequencies (about one thousand cycles per second), using a conventional Wheatstone bridge and a conductivity-cell having platinum electrodes dipping into the solution.

The high-frequency method uses any beaker or boiling-tube as the conductivity-cell, with *external* band electrodes, or with no electrodes at all. The equivalent electrical circuit of such a cell, filled with the electrolyte solution, is shown in Fig. 1.

C_1 and R_1 are the capacitance and resistance, respectively, between 'images' of the electrodes inside the cell, through the solution; the dielectric of capacitor C_1 is thus the solvent. C_2 is the resultant capacitance between the electrodes and their images, through the glass wall of the cell; the resistance of this path is so high that it may be neglected. C_2 will offer a very high impedance to the flow of low-frequency electric current between points A and B, so that we must connect the cell to a radio-frequency generator if the current is to be influenced by the cell contents. At frequencies of a few Mc/s., the impedances of C_1 and C_2 become

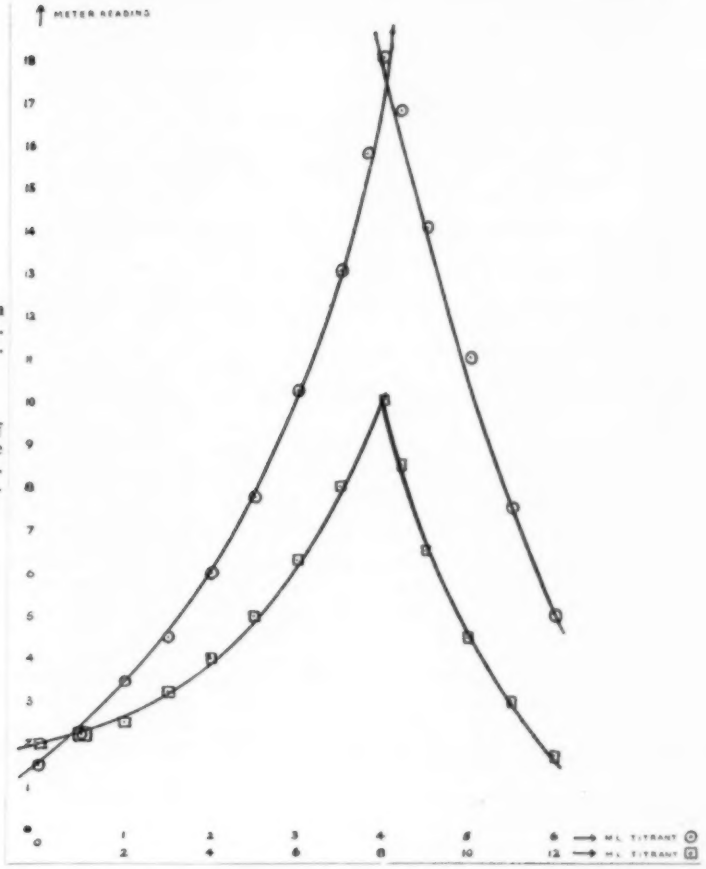
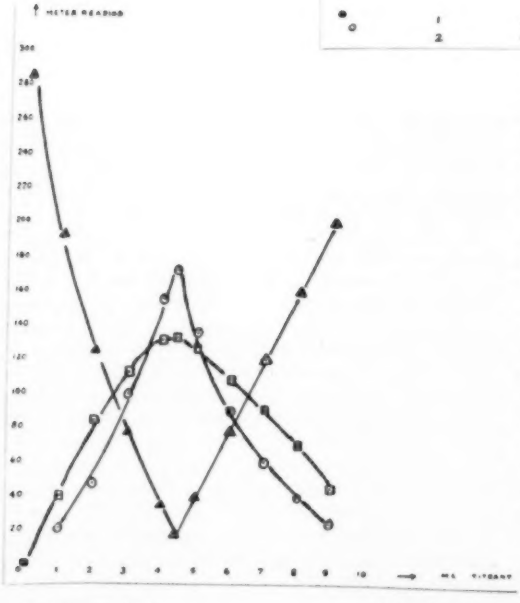


Fig. 2(*a*). Effect of concentration upon sensitivity: ○ 10 ml. N/100 NaOH v. N/40 HCl. □ 10 ml. N/20 NaOH v. N/16 HCl.

Fig. 2(*b*) (below). Effect of change of frequency upon the shape of the titration curve: 5 ml. H_3PO_4 v. approx. N/5 NaOH, ○ 10 Mc/s. □ 20 Mc/s. △ 30 Mc/s.



comparable, and the radio-frequency current varies with R_1, i.e. with the conductance of the solution. The advantages of this technique are, firstly, the absence of internal electrodes, which might become fouled by a precipitate formed in the cell, or give rise to polarization errors. Secondly, at least for comparative purposes, the measuring circuit becomes very much simpler; we can dispense with the Wheatstone bridge and measure changes in anode current or in oscillator frequency. A variety of circuits has been described in a recent review[1].

Radio-frequency conductivity measurements were first applied to the location of titration end-points by Blake[2], and by Jensen and Parrack[3] in 1946. Since then, many titrations have been followed in this way—acid-base, argentometric, oxidation-reduction—in aqueous and in mixed solvents.[1] The shape of the titration curves depends upon both frequency and concentration as seen from Fig. 2. In general, the frequency is raised in order to maintain sensitivity at higher concentration.[4] The method has mostly been used for such comparative applications, but as Reilly and McCurdy have shown[5] it is possible to 'calibrate' the cell, and hence to measure absolute conductances.

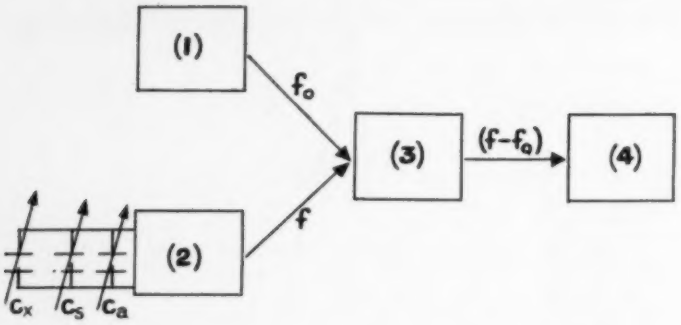


Fig. 3. Block diagram of beat-frequency oscillator: (1) fixed-frequency oscillator; (2) variable-frequency oscillator; (3) mixer; (4) beat indicator.

Dielectric Constant

We may define the dielectric constant of a medium X (gas, liquid or solid) as the ratio C_x/C_o, C_x being the capacity of a condenser with X between the plates, and C_o that of the same condenser with a vacuum in place of X. The dielectric constants of gases, and of some liquids, do not differ greatly from unity—the value assumed for the dielectric constant *in vacuo*—so that very accurate comparison of the capacities C_x and C_o is required. For this reason the measuring condenser is made part of a radio-frequency resonant circuit, and the capacities are found by the 'heterodyne beat' method, illustrated in Fig. 3.

C_x is the measuring condenser, C_s a standard variable condenser which may replace C_x in the circuit, and C_a a small variable capacitor. The two radio-frequency oscillators produce a 'difference' beat-frequency if one is slightly detuned from the other. With C_x empty, C_a is adjusted for zero beat-frequency; C_x is replaced by C_s, and zero beat is restored, i.e. $f = f_o$. The value of C_s is noted, and the experiment repeated with the liquid under test in C_x; if the new value of the standard condenser is $C_s' : C_s'/C_s = C_x/C_o$, due allowance being made if necessary for the dielectric constant of air.

If two miscible liquids differ considerably in dielectric constant, mixtures of them may be analysed by measuring this property. Thus, mixtures of ethyl alcohol and water—the components having dielectric constants of 24·2 and 78·5 respectively, at 25°C.—may be analysed in this way. The moisture content of various powdered materials, and of organic liquids, may be accurately estimated, because of the high dielectric constant of water; the powdered materials are packed between the plates of the measuring condenser. The separation of the components of a mixture by fractional distillation may be similarly followed.

The high-frequency conductivity apparatus already described can also follow changes in dielectric constant, so that, with a suitable calibration curve, the analysis of mixtures is possible.[1] In this case, variation in C_1 rather than in R_1 (Fig. 1) is followed.

Dipole Moments

The dielectric constant of a medium is a *bulk* property to which all the molecules present contribute; the related property of the individual molecules is the *dipole moment*, and interpretation of dielectric constant is mainly in terms of this quantity.

In a diatomic molecule AB the electrons which form the covalent bond are always unequally shared, unless A and B are identical; the resulting unbalance of electric charge forms an electric dipole. In a complex molecule each bond makes a contribution, and the resultant is obtained by vectorial addition of the bond dipoles. In an applied electric field the molecules tend to turn so as to align their dipoles with the field, because this is the state of minimum energy; the molecules are said to undergo *orientation polarization* P_o. In addition, the electric field distorts the molecule by its interaction with the electrons and the atomic nuclei; this is the *distortion polarization*, P_d. The overall effect of the field is the *total polarization*, P, which is related to the dielectric constant ε by the Debye equation:

$$\frac{\varepsilon - 1}{\varepsilon + 2} \cdot \frac{M}{d} = \frac{4\pi N}{3} \cdot P \qquad \ldots (1)$$

an equation which applies strictly to dilute (i.e. low-pressure) gases, but is a good approximation for *dilute* solutions of polar solutes in non-polar solvents such as benzene. In this equation, M is the molecular weight, d the density, and N is Avogadro's number.

If, then, we measure the dielectric constant in the manner already described we can find P. The component of P which is of immediate interest is P_o, because the orientation of the molecules depends upon the charge-separation, i.e. upon the *dipole moment* (charge × distance of separation). At a frequency of about 10^6 cycles per second the molecules are able to follow the alternations of the field, and equation (1) applies. At higher frequencies, however, the molecules can no longer 'keep in step' with the field, and part of P_o is lost (Fig. 4). Eventually, as the frequency is increased still further the molecules remain practically at rest, and only the component P_d of P is left as the polarization. We cannot measure dielectric constants at such high frequencies, but we can easily measure a related property, the *refractive index n*. Maxwell showed that if

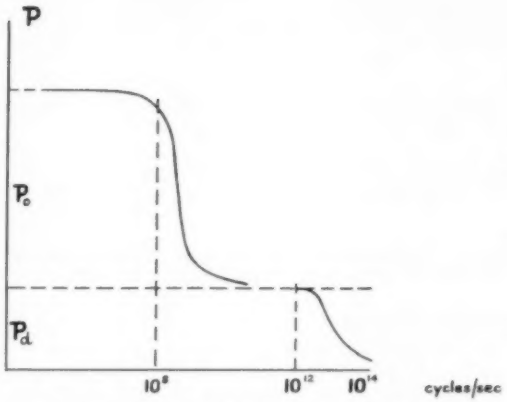


Fig. 4. Variation of total polarization with frequency.

we could measure ε and n at a common frequency f, we could relate them by: $\varepsilon_f = n_f^2$.

If we measure n at the frequency of the yellow sodium lines, P_o is reduced to zero, and equation (1) becomes:

$$\frac{n^2 - 1}{n^2 + 2} \cdot \frac{M}{d} = \frac{4\pi N}{3} \cdot P_d \qquad \ldots (2)$$

Subtraction of P_d from P gives P_o, which is related to the dipole moment μ by:

$$P_o = \frac{4\pi N}{9kT}\mu^2, \text{ or: } \mu = 0{\cdot}01273\sqrt{P_oT} \times 10^{-18} \text{ e.s.u.}$$

In dealing with *solutions*, equation (1) is modified:

$$P_2x_2 = \frac{\varepsilon_{12} - 1}{\varepsilon_{12} + 2} \cdot \frac{M_1x_1 + M_2x_2}{d_{12}} - P_1x_1$$

x_1 and x_2 are the molar fractions of solvent and solute, P_1P_2 their total polarizations, and M_1M_2 their molecular weights; ε_{12} is the dielectric constant of the solution, and d_{12} its density. For a non-polar solvent, P_1 can be measured directly; the P_2-values are extrapolated to infinite dilution to eliminate the interaction of the solute molecules. Subtraction of the distortion polarization (calculated in a similar manner from the refractive index) enables the dipole moment of the solute molecule to be calculated.

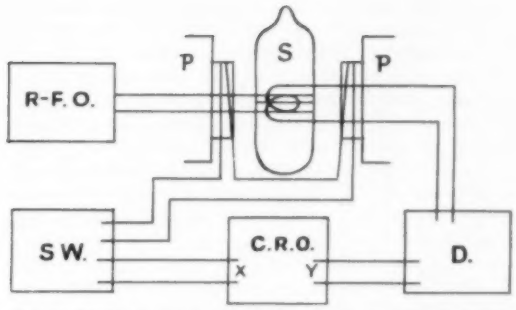


Fig. 5. Block diagram of nuclear magnetic resonance spectrometer. S, sample. R-F O, radio-frequency oscillator. SW, sweep generator. D, detector. C.R.O., cathode ray oscilloscope. PP, magnet poles.

Nuclear Magnetic Resonance Spectroscopy (NMR)

This very important technique depends upon the joint effect of a magnetic and an electric (radio-frequency) field upon those atomic nuclei which possess a magnetic moment. In Fig. 5, the block diagram of a typical NMR spectrometer is shown. The sample (liquid or solution) is hung in the 5 cm. gap between the pole pieces of the magnet; the field-strength is about 14,000 gauss. A source and a detector of radio-frequency oscillations are inductively 'coupled' to the sample. At certain combinations of field-strength and oscillator frequency the nuclear magnetic dipoles absorb power, and a 'spectrogram' is obtained, rather as in infra-red spectroscopy.

In practice the frequency is kept constant and the field-strength is varied by means of auxiliary coils wound on the pole-pieces, as shown. The coils are fed from a sweep generator synchronized with the recorder, e.g. supplying the time-base of a cathode ray oscilloscope. The oscillator coil is perpendicular to the lines of the magnetic field and also to the detector pick-up coil, so that coupling between oscillator and detector is *via*

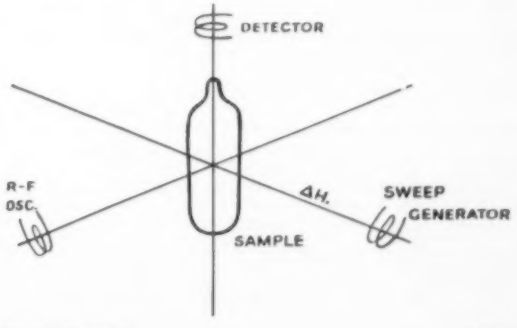


Fig 6. Coupling of detector and oscillator coils through the sample.

magnetic dipoles induced in the sample and in a direction perpendicular to the axis of the oscillator coil. (Fig. 6.)

Atomic nuclei fall into three 'magnetic' classes:

(1) those having no nuclear spin and therefore zero magnetic moment, and also having a uniform charge-distribution and so zero electric quadrupole moment. Such nuclei are ^{16}O and ^{12}C, and they show no NMR spectrum. (This latter is an actual *advantage*, in studying organic compounds, because it does not interfere with the proton spectrum; ^{13}C has a magnetic moment, and hence enrichment enables us to study the carbon spectrum where necessary.)

(2) those nuclei which possess nuclear spin and magnetic moment, but again have zero quadrupole moment, e.g. ^{1}H, ^{13}C, ^{15}N, ^{19}F.

(3) some nuclei may be represented as prolate or oblate spheroids, possessing both a magnetic moment and a quadrupole moment. Examples, are ^{2}D, ^{14}N; and ^{17}O, ^{35}Cl, respectively. The spectra of such nuclei are more complex, but yield more chemical information.

If the nuclear spin is I, the component m of magnetic moment along a given direction is 'quantized', that is, limited to a discrete set of values:

$m = I, (I - 1), (I - 2), \ldots O \ldots -I.$

E.g. if $I = 1/2$, $m = +1/2$ or $-1/2$.

if $I = 1$, $m = 1$, O or -1.

For $I = 1/2$, in the absence of any disturbance, just as many nuclei will have $m = +1/2$ as have $m = -1/2$. An applied magnetic field, however, causes these two orientations to differ in energy, with $m = +1/2$ (alignment *with* the field) representing a lower energy than the alternative orientation $m = -1/2$. However, as in the case of electric dipoles, the tendency for an 'ordered' arrangement is disturbed by thermal agitation; for protons in a field of 10,000 gauss, the excess of nuclei with $m = +1/2$ is only about 0·001 per cent at room temperature, increasing to 0·003 per cent at liquid air temperature.

The energy required by the nuclei to 'flip over' from $m = +1/2$ to $m = -1/2$ is drawn from the radio-frequency field, and this absorption of energy is indicated by the detector. The frequency at which absorption occurs is related to the field-strength by:

$f = \mu.H$, where μ is the nuclear magnetic moment, measured in terms of a fundamental unit, the Bohr magneton. Hence for a given applied frequency the field-strength may be varied until this relationship holds, when absorption will occur. Typical values of the frequency for some common nuclei are:

Nucleus	*I*	*f, Mcs./10⁴ gauss*
^{1}H	1/2	42·6
^{2}H	1	6·5
^{13}C	1/2	10·7
^{14}N	1	3·1
^{15}N	1/2	4·3
^{17}O	5/2	5·8
^{19}F	1/2	40·1
^{31}P	1/2	17·2

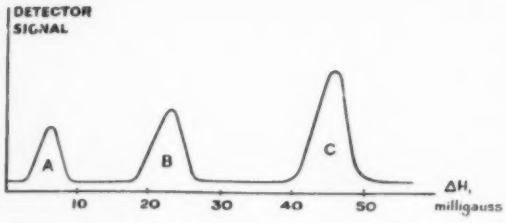


Fig. 7. Low-resolution NMR spectrum of ethanol; proton resonance. A, -OH peak; B, $-CH_2-$ peak; C, $-CH_3$ peak.

Applications of NMR

The low-resolution NMR spectrum of ethanol is shown in Fig. 7; this dispersion has been produced by a variation of about 50 *milligauss* in an average field-strength of 9·4 kilogauss, illustrating the constancy of field which is required in NMR. The peaks are due to proton resonances; evidently three 'kinds' of protons are present, differing in their environments, and of relative abundance (proportional to the areas under the peaks) 1 : 2 : 3. These correspond to hydroxyl-, methylene-, and methyl-group protons, an interpretation which is confirmed by comparison with the NMR spectrum of C_2H_5OD. The -OD resonance, at this frequency, would occur at 58 kilogauss, and so only two peaks appear.

It is this sensitivity to environment, i.e. to the screening of nuclei from the field by their surrounding electrons, which causes each molecular species to give a characteristic NMR spectrum. For comparison purposes, such resonance lines are reported in terms of a parameter δ,

$$\delta = \frac{H_s - H_R}{H_R} \times 10^6$$

where H_s is the field-strength at which the sample shows absorption, and H_R the field-strength at which a reference substance shows absorption in the same radio-frequency field. The reference substance is chosen to show one sharp line for the nucleus considered; thus, for proton resonance, water, benzene, and cyclohexane are suitable.

Some typical δ-values are:

$-CO_2H^*$	−6·4	H_2O	0·0 (ref.)
$R-OH^*$	−0·1	$R-O^*NO$	−170
H^*OH	0·0 (ref.)	$R-CO_2{}^*H$	−220
$-CH_2{}^*-$	3·5	$R-ONO^*$	−380
$-CH_2-CH_3{}^*$	4·1	$C=O^*$	−600

*atomic nucleus concerned.

NMR can also be used in quantitative analysis because the area under the resonance peak will be proportional to the number of nuclei involved provided that there is no marked solvent interaction. Thus,

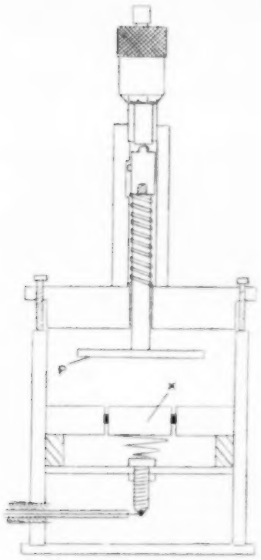


Fig. 8. Diagram of acoustic interferometer. Y, piezo-electric crystal. P, moveable plate.

the ratio of the areas under C_6H_6 and C_6H_{12}-proton peaks should give the ratio of benzene to cyclohexane in a mixture of these. Direct comparison of peak-heights is only possible if the resonance peaks are of the same width.

Acoustic Interferometry

In our last technique, radio-frequency oscillations are used to drive a crystal plate X, which is in contact with a liquid or solution. The crystal is part of an interferometer (Fig. 8); it vibrates at its natural frequency f_x and sets up longitudinal waves in the liquid. The moveable plate P is slowly raised through the solution on a micrometer screw; as it passes nodal positions, a standing-wave system is set up, and power is transferred to a sensitive detector. If the distance apart of successive nodes (the half-wavelength) is $\lambda/2$, the velocity of the 'sound' wave, c is given by: $c = 2f_x \cdot \lambda/2$.

In general, c increases as the concentration of the solution increases; sensitivity of measurement is maintained up to very high concentration, so that the analysis of electrolyte solutions close to saturation is possible.

In dilute solutions, $\lambda/2$ does not change very much from its value in the pure solvent, and it is essential that the frequency of the sound-wave generator should remain constant. It is well known that a piezo-electric crystal, driven at a frequency within the range we are considering, maintains constancy to within about 1 part in 10^6; and this gives a final illustration of an analytical technique dependent upon radio-frequency.

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Correct as at June 1, 1961.

HIGH-VOLTAGE PAPER ELECTROPHORESIS

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Introduction

Paper electrophoresis has become established over the last few years as a most useful analytical and preparative technique. The literature on the subject has grown to such an extent that a recently published book contained more than 3,000 references.

There is an outward similarity between this technique and paper chromatography in that both are separation techniques, using the same supporting medium and similar methods of detection and evaluation. The fundamental difference between them, however, is that each is based on an entirely different physico-chemical principle leading to a different migratory behaviour. This means that compounds that cannot be separated by chromatography can often be separated by electrophoresis, and vice versa. In electrophoresis, separation of the constituents of a mixture depends on the differing mobilities of ions in an electric field, in chromatography separation depends on differences in the partition or adsorption characteristics of individual constituents.

Broadly speaking, paper chromatography was designed and is still more useful for low-molecular compounds, whilst the reverse holds true for paper electrophoresis. In fact, the latter is used overwhelmingly in the field of proteins, particularly serum proteins, and its application to low-molecular compounds has made rather slow progress.

It appears that with low-molecular compounds the advantages of electrophoresis are not sufficiently great and convincing to induce wider-spread adoption of the technique. A comparison with a corresponding paper chromatographic method shows that, apart from a usually different sequence of positions, the time taken for a run is of the same order, i.e. 16-20 hr., and the quality of spots, i.e. shape and size, certainly not better, sometimes even inferior. This is due to diffusion effects which tend to enlarge spots or bands, blur sharp boundaries between adjacent zones and make resolution of compounds of similar mobilities very difficult.

Proteins, large colloidally dispersed molecules with relatively high electric charges under suitably chosen conditions, have mobilities of the same order as most low-molecular ions, but much lower diffusion coefficients so that even after a run of 16 hours the zone boundaries are sufficiently sharp and resolution is satisfactory. It is therefore obvious that any improvement in the efficiency of the technique for small molecules can only be brought about by a significant reduction in the time taken by the ions to travel the required distances on the paper sheet. In other words, to make the time of experiment so short that diffusion would not interfere with clean separations.

D. Gross, Ph.D.

Theoretical Considerations

The migration of an ion in paper depends on many conditions such as magnitude and sign of its net charge, ionic strength, pH, conductance, temperature, viscosity of surrounding electrolyte, electro-osmosis, adsorption etc. But assuming that all these factors are the same, the migration is proportional to the field strength applied. The ion can thus be made to migrate at a higher rate within a given time by increasing the potential applied to the path of migration. Mobility of an ion can be expressed:

$$\text{Mobility} = u = \frac{d/t}{V/l} \qquad (1)$$

$$\text{Field Strength} = X = \frac{V}{l} \qquad (2)$$

$$d = \frac{u V t}{l} \qquad (3)$$

where V = voltage applied, l = length of field, d = distance travelled by ion, t = time.

It is thus clear according to (3) that doubling the field strength will lead to halving the time of run for a given distance travelled. But at the same time the electric current flowing through the moist filter paper increases according to Ohm's law:

$$\text{Current} = I = \frac{V}{R} \qquad (4)$$

where R = electrical resistance of paper strip.

The flow of current generates heat and the heat evolved, in calories per second, increases not proportionally to but with the square of the current, and is given according to Joule's law:

$$\text{Heat} = Q = \frac{RI^2}{A} = \frac{VI}{4{\cdot}185}$$

where A = mechanical heat equivalent
$= 4{\cdot}185 \cdot 10^7$ erg/cal.

Increased heat generation leads to immediate trouble with conventional low-voltage apparatus (3-5 V./cm.), because there the dissipation of heat is effected mainly by evaporation, which can be tolerated only within very narrow limits, say, up to 0·005 W./cm.^{2}, without seriously interfering with the quality and reliability of results. Excessive evaporation causes erratic behaviour of the ions and makes measurement of mobilities impossible. It is also imperative to prevent the temperature in the strip from rising above 30°C. As both diffusion and conductance are temperature-dependent, the interfering influence of diffusion becomes progressively stronger and the increasing conductance causes a rise in current consumption and thus more heat generation. The process runs out of control and a vicious circle is set up that cannot be broken. If on the other hand, all generated heat can in some way be effectively dissipated, then there is no limitation to the voltage applied. The feasibility of high-voltage techniques becomes thus mainly a problem of efficient cooling. If the temperature is not allowed to rise, optimum conditions can be chosen and strictly controlled, with attendant benefits such as good reproducibility, high resolving power and safe operation. By measuring the migration rates of ions in relationship to time and field strength, the degree of control of steady conditions can easily be ascertained. Under strictly controlled temperature and flow conditions there should be a linear relationship, according to (3), between distance travelled and temperature or voltage applied.

The voltage and current level is limited only by the capacity of apparatus to dissipate the heat, preferably by conduction only.

Apparatus

The various designs of high-voltage equipment (for 50 V./cm.) described can be reduced to three, according to the basic principles of cooling applied:

(1) by liquid heat-exchanger, using a suitable organic solvent,

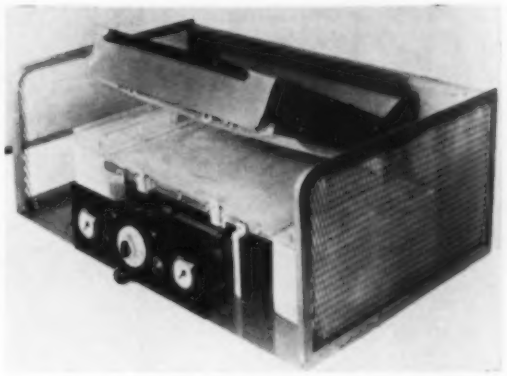


Fig. 1. Front view of high-voltage electrophoretic apparatus, opened position.

(2) by solid heat-exchanger, with only the bottom of the paper strip in contact with the cooling surface,

(3) by solid heat-exchanger, with both top and bottom in contact with cooling surfaces and under carefully controlled pressure.

A design according to (1) was first reported by Michl[1] and widely adopted because of its relative simplicity, low cost and usefulness. Several modifications[2,3] have since been described, introducing greater volumes of solvent and various ways of cooling it, either by tap water or refrigerated brine. The principle is the same, viz. the buffer saturated strip is immersed between two electrodes in a large volume of water—immiscible organic solvent such as toluene, heptane, etc., which acts both as a heat-conductor and insulator. No evaporation takes place and heat dissipation up to 0·2 W./cm.^{2} is attainable. The disadvantages are possible solubility and reaction of migrating ions with the solvent. In addition, a certain amount of solvent is evaporated in the process, introducing toxic and fire hazards.

Principle (2) was applied in several instruments described[4,5], with one notable variation. Werner and Westphal[6] deliberately leave a headspace of a few mm. between strip and glass plate, thus creating a very small 'moist chamber' where cooling is apparently meant to be helped by a certain amount of evaporation. Whether this compromise solution is of great benefit is highly doubtful. The normal procedure is to place the paper strip on the cooled (by refrigerated brine) glass or plastics base plate and cover it loosely with a glass plate. Dose[5] uses an electrically insulated copper plate for better heat conduction. The cooling capacity of this type of design is about 0·1 W./cm^2.

It is evident that greater cooling efficiency is afforded by an apparatus based on principle (3). Such an instrument was first suggested by Michl[7] and Gross[8]. The latest equipment of this type developed over a number of years, was recently described in detail by Gross[9]. This design, where the paper strip is sandwiched between two efficiently cooled, electrically insulated

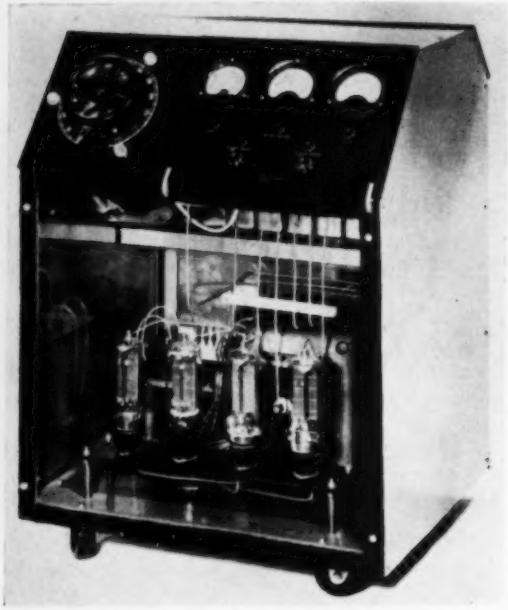


Fig. 2. Power unit capable of supplying 500 mA. at 5 kV.

(polythene film) aluminium plates has a capacity for dissipating energy of up to 1 W./cm.^{2}, and entails for successful operation application of closely controlled pressure. The solution to the latter problem was found in a relatively simple pneumatic device exerting highly controllable and reproducible pressure on the paper strip. Figs. 1 and 2 show the apparatus and the power unit. Particulars of construction can be obtained from the above mentioned paper[9].

The advantages of the apparatus are quality and high reproducibility of results, great resolving power and versatility, although high-voltage electrophoresis is, for various reasons, not applicable to proteins. Tap water of an average seasonal temperature of 12°C. can be satisfactorily used as coolant, thus dispensing with the need for an expensive refrigerating unit. A disadvantage is the fairly high cost of construction, attributable mainly to the need for precision-ground cooling plates and a power unit of high capacity. The latter is indispensable when using a paper strip, 30 cm. wide. This width considerably extends the scope of work, e.g. facilitating two-dimensional high-voltage electrophoresis[10]. The flow of liquid in the paper strip, due to capillary suction, evaporation and electro-osmosis and causing irregular patterns of separation, can be well controlled by interposing Cellophane barriers (sleeves) between the strip and the electrode vessels[11]. Cellophane allows the current to pass but severely restricts the influx of electrolyte from either buffer vessel into the strip. This, and the fact that the strip is compressed between the cooling plates, prevent undesirable movement of liquid during the comparatively short period of the run. An illustration of the evident effect of Cellophane barriers on the flow pattern is presented in Fig. 3.

Safety of operation is ensured by suitably positioned current cut-out devices and by placing the instrument in a safety-cage allowing constant visual inspection without any risk to the operator. No accident has occurred in this laboratory in nine years of almost daily operation.

Results

High Voltage v. Low Voltage.—The question is often raised whether the results of the high-voltage technique justify the far greater initial expense of more elaborate equipment. The answer is amply demonstrated by Figs. 4 and 5.

Fig. 4 shows a comparison of two separations of the same mixture of polyhydric alcohols carried out under conditions of low voltage and high voltage. The conditions were chosen so that the lower voltage in one case was balanced by a correspondingly longer running time. As the strip was 50 cm. long, the applied potential gradients were 7 V./cm. and 100 V./cm. respectively.

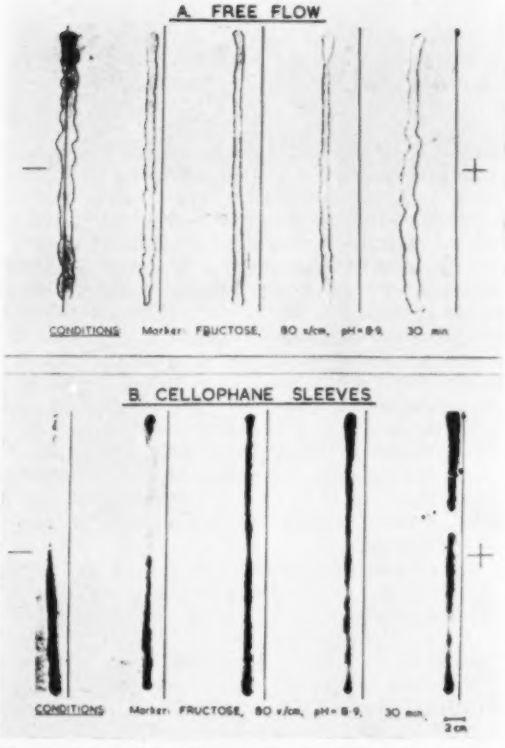

A FREE FLOW
CONDITIONS Marker: FRUCTOSE, 80 v/cm, pH = 8·9, 30 min
B CELLOPHANE SLEEVES
CONDITIONS Marker: FRUCTOSE, 80 v/cm, pH = 8·9, 30 min
2 cm

Fig. 3. Pattern of liquid flow during electrophoresis, with and without the use of Cellophane barriers (sleeves).

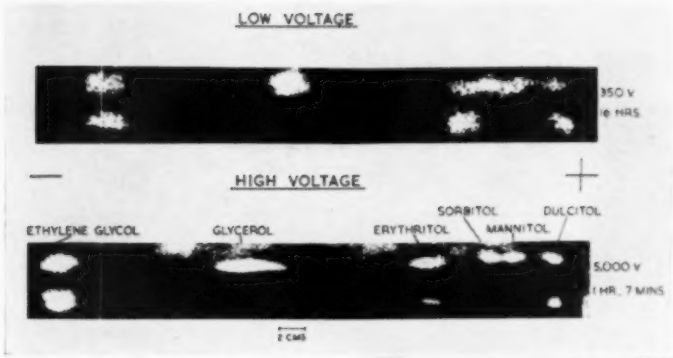


Fig. 4. Electrophoresis of polyhydric alcohols at high and low voltage in 0·05M sodium borate buffer of pH 9·2.

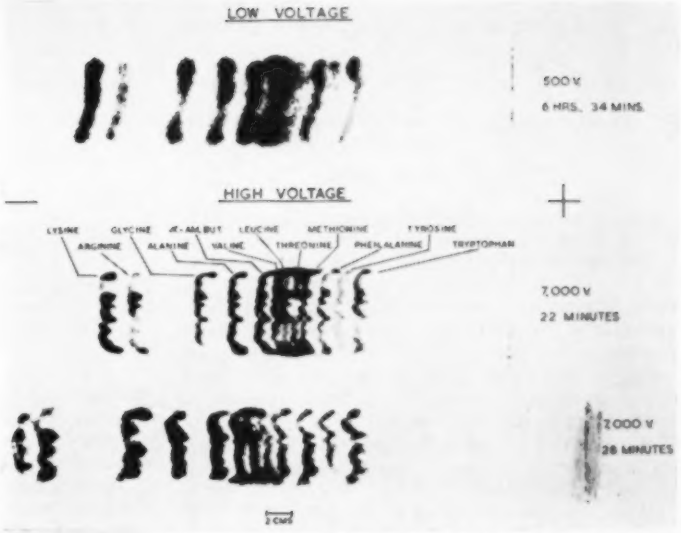
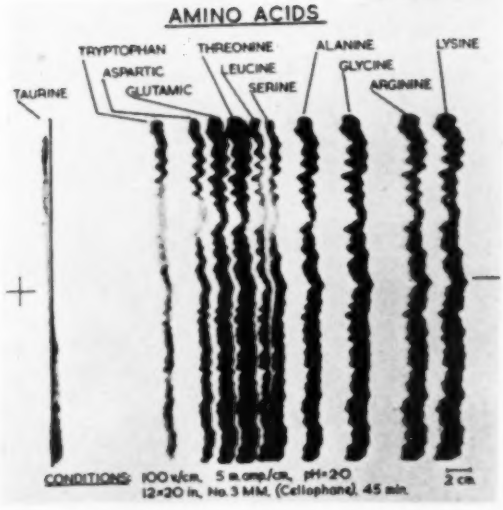


Fig. 5. Electrophoresis of amino-acids at high and low voltage in 0·75M formic acid of pH 2·0.



Two advantages afforded by the high-voltage technique are immediately evident. The compactness of the spots, due to lack of diffusion effects, the clean separation of the series erythritol to dulcitol, and the correct position of ethylene glycol, unaffected by irregular flow of electrolyte. The separation of sorbitol from mannitol is possible only by use of high voltage, owing to the very small differences in mobilities[1,2].

A similar demonstration of the superiority of high voltage is given in Fig. 5, dealing with amino-acids.

The choice of experimental conditions was based on the same idea as in Fig. 4, with potential gradients of 10 V./cm. against 140 V./cm. It is obvious that the low-voltage separation lacks sharpness and definition, particularly with regard to the series valine to methionine, whereas the high-voltage electrograms show a good resolution of the mixture.

It must be added that the results for Figs. 4 and 5

Fig. 6. Electrophoresis of wide bands of amino-acids in 0·75M formic acid of pH 2·0.

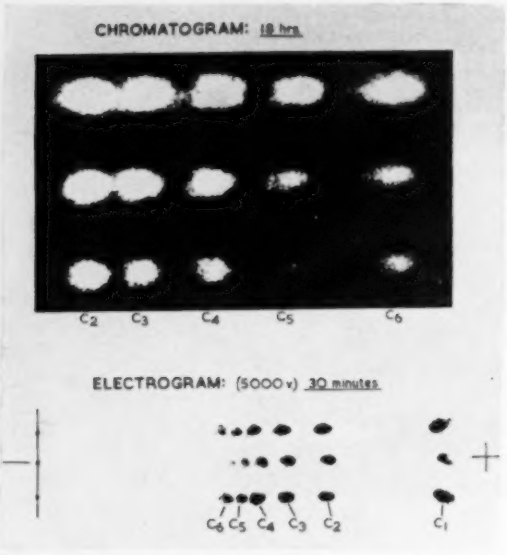


Fig. 7. Comparison of high-voltage electrophoresis, in approx. 0·1 M ammonium carbonate sol. of pH 8·9, with paper chromatography of lower fatty acids (C_1-C_6).

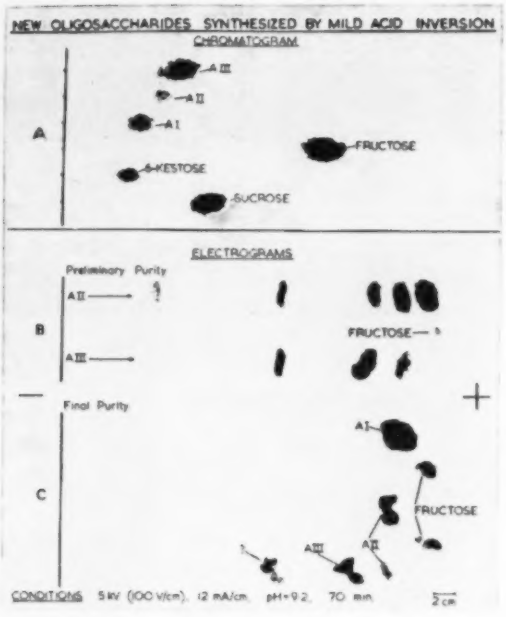


Fig. 8. Comparison of high-voltage electrophoresis, in borate buffer, with paper chromatography of new oligosaccharides synthesized by mild acid inversion of sucrose.

were obtained on a smaller model which, though in principle of the same design, was not quite capable of producing the same overall quality of separated zones, owing to lack of width and consequent edge effects. Fig. 6 shows a separation of a similar mixture of 11 amino-acids by the larger model.

It is interesting to note that the mixture contains up to 1 mg. of amino-acids, which indicates potential preparative applications.

High Voltage vs. Chromatography.—The short running time and high resolving power offer definite advantages, in comparison with some paper chromatographic procedures. Fig. 7 illustrates a comparison between the times required and results obtained in the case of a separation of lower fatty acids, C_1-C_6, of great importance in many branches of research and industry.

Whereas by chromatography it takes 18 hours to produce a reasonably good separation, without resolving C_1 and C_2, the electrogram shows a sharp separation of all six acids, after 30 minutes at 100 V./cm. Of particular interest is the easy and unmistakable resolution of formic acid and acetic acid, which can actually be done in only 10 minutes. Acids up to C_{10} can be separated by the same procedure[13].

An interesting example of the superior resolving power of the high-voltage technique is demonstrated in Fig. 8.

In a recent basic investigation into mild inversion products of sucrose, chromatography was extensively used to isolate the new products which after twice repeated chromatographing appeared to be 'homogeneous' and of clearly reproducible R_f values. The chromatogram on top of Fig. 8 shows the closeness of the positions of products AI to A III, wedged between 6-kestose and sucrose. No better resolution by variation of the chromatographic procedure was to be expected. However, when electrophoresis in borate buffer was applied, the picture that emerged was quite startling.

AI appeared to consist of a major and a minor

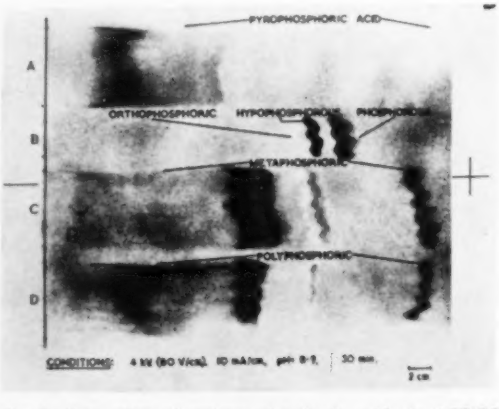


Fig. 9. Separation of various phosphoric acids in 0·75 M formic acid of pH 2·0.

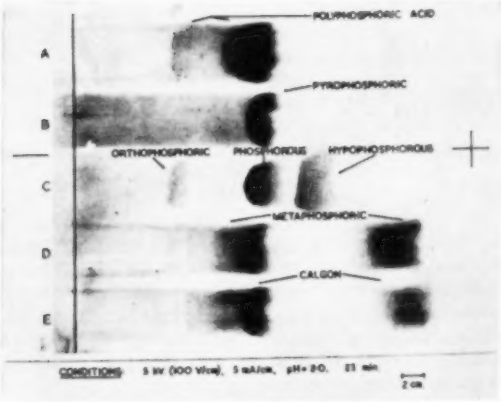


Fig. 10. Separation of various phosphoric acids in approx. 0·1 M ammonium carbonate solution of pH 8·9.

component (not shown), AII and AIII were mixtures of up to five components.

After subjecting the products to further chromatographic purification, products were finally obtained which, as far as AI and AII were concerned, appeared to be single compounds, with AIII still containing some AII and an unidentified component. It would thus appear that in some cases electrophoretic 'homogeneity' is a more critical standard than that afforded by a chromatographic procedure.

Complex Mixtures.—Many complex mixtures, including inorganic ions[14], amino-acids and other organic acids[10], have been shown to be resolvable by the high-voltage technique. Its application to the problem of separating the various oxy-acids of phosphorus is illustrated by Figs. 9 and 10.

Fig. 9 shows the results of a separation at pH 2·0, giving a clean, unambiguous resolution of orthophosphoric, phosphorous and hypophosphorous acids. It also shows the inhomogeneity of the other acids.

In Fig. 10 a pH value of 8·9 was chosen, affording a slightly inferior resolution of the three first-named acids together with an interesting reversal of sequence, but a sharper resolution of the other acids. Unfortunately, the ammonium molybdate reagent used spoiled the background somewhat. It gave, however, a characteristically lighter colour for orthophosphoric acid.

Conclusions

Recently developed high-voltage paper electrophoretic techniques, applying potential gradients of 50V./cm. have made paper electrophoresis a far more useful and interesting analytical tool, thanks to the significantly increased resolving power and greatly reduced time of running. In some cases, a clear superiority over paper chromatographic methods, both in time and results, has been established. The possibility of obtaining results in a matter of minutes instead of hours or days, is a great all-round stimulating factor both in research and routine work. The versatility of the technique has been demonstrated in various fields of inorganic and organic chemistry, except in that of proteins. The requirement of elaborate and somewhat expensive equipment is the only obstacle to a wider-spread adoption of the technique. The possibility of possessing a powerful tool, one often complementary to chromatography, should nevertheless prove in the long run a convincing argument for adopting it. Experience has shown that scientific progress is closely linked with the acquisition of new or more reliable data. If such data can be obtained by the use of novel instruments or techniques, high initial cost of equipment will never be too serious an obstacle.

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New British Chemical Standards

Bureau of Analysed Samples Ltd. announce the issue of the following new British Chemical and Spectrographic Standard Samples:

B.C.S. No. 300 6 per cent zinc aluminium alloy analysed for Cu, Mn, Si, Mg, Fe, Ti, Cr and Zn.

B.C.S. No. 304 10 per cent aluminium bronze analysed for Cu, Zn, Ni, Fe, Mn, Si and Al.

B.C.S. No. 305 75 per cent ferro-silicon analysed for P, Al, Ca and Si.

B.C.S. No. 306 0·4 per cent carbon free-cutting steel analysed for Si, S, P, Mn and C.

The following samples are only available in the finely divided form for chemical analysis:

S.S. Nos. 21-24 A series of low tungsten steels standardized for tungsten only with tungsten contents ranging from 0·7 per cent to 3·4 per cent.

This series is supplied in the form of $\frac{3}{4}$ in. diameter rods each 3 in. long for use as Spectrographic Standards, but is also available in the form of turnings for chemical analysis bearing the reference numbers B.C.S. 281-284.

Further details of these new samples are given in List No. 400R and the latest supplementary insert sheet, which are obtainable free on request from Bureau of Analysed Samples Ltd., Newham Hall, Middlesbrough.

ORIGINAL CONTRIBUTION

THE DETERMINATION OF METALLIC ZINC BY MEASUREMENT OF HYDROGEN EVOLUTION

by G. Lindley

Works Chemical Laboratory, English Electric Co., Ltd., Bradford

Introduction

During the course of investigations into the effects of heat on the oxidation rate of powdered zinc, it became necessary to determine the metallic zinc contents of zinc-zinc oxide mixtures. As many determinations were involved it was decided to try to develop a simple and rapid method based on the measurement of the volume of hydrogen evolved from dilute acid. Under these conditions no hydrogen would be evolved from the zinc oxide.

An apparatus was required in which the reaction vessel and gas burette were an integral unit so that a simple construction could be attained, particularly with regard to temperature control. An arrangement was also required whereby the sample could be introduced into the dissolving acid without disturbing the initial equilibrium of the apparatus. The apparatus finally constructed is described below, and its application to the determination of zinc is illustrated by results obtained on pure zinc. The principle could also be employed where a stoichiometric or reproducible evolution of a simple gas is involved in other reactions.

Construction

Details of construction may be altered to suit particular requirements.

To cover zinc contents up to 100 per cent, using a 0·1 g. sample, hydrogen volumes up to about 37 ml. at S.T.P. had to be measured. This represented a suitable sample weight and gas volume. By using a normal 50 ml. burette, sufficient accuracy was attained, as Class A and B tolerances are ± 0·06 and ± 0·1 ml. respectively, corresponding to ± 0·17 and ± 0·29 per cent Zn. on the 0·1 g. sample. As the method was for zinc contents above about 20 per cent, the measuring errors were considered to be sufficiently small.

The apparatus was constructed as follows:

The stopcock was cut off the bottom of the burette (A) (Fig. 1) and a length of glass tubing was sealed on. After subsequent insertion of the burette assembly into the lower bung of the water jacket, this tube was bent and connected by rubber tubing to the levelling bottle (B). A tube of about 30 mm. diameter and about 100 mm. long (C) was joined to the top of the burette, and at the joint three indentations (X) were made in the glass. During glass-working on the burette care was taken to use only a small flame to localize the heat and minimize errors in the calibrations. The burette unit was mounted in the water jacket (D) fitted with filling and vent tubes (E).

The reaction vessel (F) was a tube about 25 mm. diameter and 50 mm. long to the top rim of which was welded a small glass loop. With a simple hook this tube and its contents could be lowered into or withdrawn from the burette. The glass sample tube (G) about 10 mm. × 20 mm. carried a thin glass extension hook as shown.

A bent phosphor bronze strip (H) was soldered to the end of a short brass rod (I). To the ends of the strip were soldered thin sheet steel tips (J). A hole was drilled through one of these tips and the other was formed into a thin hook. In the normal closed position the springy strip maintained the hook through the hole, and the sample tube could be hung on this hook. On

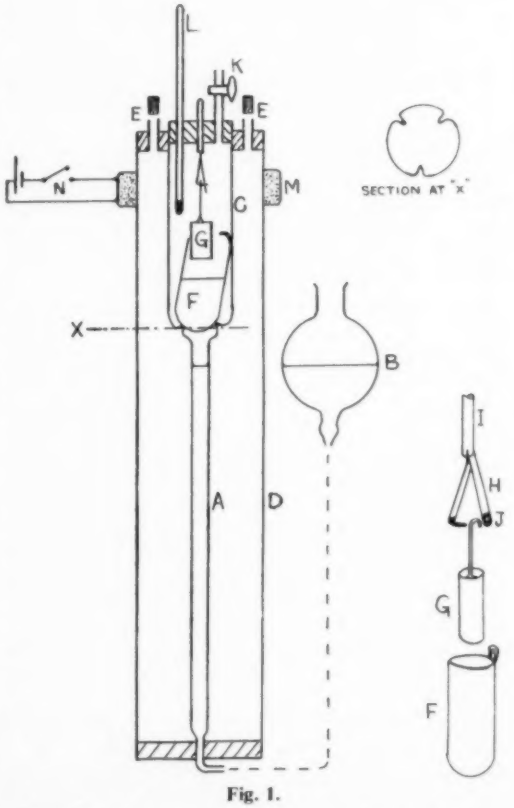

Fig. 1.

magnetization, from the coil (M) mounted on the water jacket, the steel tips repelled each other, thus allowing the sample tube to fall into the reaction vessel. To avoid corrosion from any acid spray, the metal parts were coated with a protective film (e.g. epoxy resin). Stopcock (K) and thermometer (L) completed the apparatus. With the above arrangements the free air volume of the apparatus was kept to a minimum.

Operation

The following example refers to the determination of metallic zinc. The burette and levelling bottle contained 5 per cent sulphuric acid coloured with a little methyl orange. For sample dissolution 15 per cent sulphuric acid containing 0·2 per cent nickel sulphate as catalyst was used. It was found that this mixture produced a rapid reaction, usually complete within a few minutes. The reaction vessel (F) was half-filled with dissolving acid, and the vessel was then lowered into the burette. A 0·1 g. sample was weighed into the sample tube which was then hung on to the release mechanism. The whole assembly was then fitted, with its rubber bung, to the top of the tube (C). Stopcock (K) was opened, the burette liquid adjusted to zero, and several minutes were then allowed for equilibration. The stopcock was then closed, and on closing switch (N) the sample was released into the dissolving acid. Gas evolution was measured until a constant reading was obtained. The sample and reaction tubes were then removed by a hook and the apparatus was ready for the next sample.

Calculation

Let V = volume of H_2 measured
T = temperature °C. in apparatus
P = atmospheric pressure
p = saturated water vapour pressure at T °C.

$$\text{Corrected volume of } H_2 = V \times \frac{273}{(273 + T)} \times \frac{(P - p)}{760}$$

One ml. $H_2 \equiv 0{\cdot}002917$ g. Zn $\equiv 2{\cdot}917\%$ Zn on 0·1 g. sample, So that per cent Zn in sample

$$= 2{\cdot}917\ V \times \frac{273}{(273 + T)} \times \frac{(P - p)}{760}$$

Results

Accuracy checks were carried out using sawings from a block of British Chemical Standard zinc of 99·989 per cent purity. First trials with the apparatus gave results of the order of 99·5 per cent Zn. To determine if any errors were involved due to solubility of hydrogen in the burette liquid, the latter was removed, saturated by bubbling the gas through for a period, then returned to the apparatus. Subsequent determinations gave somewhat more accurate results, of the order of 99·8 per cent Zn. It is considered, however, that this difference in results is not significantly due to solubility errors, but includes other errors inherent in the overall method.

Replicate determinations on the pure zinc standard gave the results in Table I.

TABLE I

*Corrected vol. of H_2**	*% Zn.*
34·2 ml.	99·8
34·2 ml.	99·8
34·3 ml.	100·1
34·1 ml.	99·5
34·2 ml.	99·8
34·1 ml.	99·5
34·2 ml.	99·8
34·3 ml.	100·1

*Rounded to one decimal place.

The results in Table I gave a mean zinc content of 99·8 per cent. Reproducibility was quite good as shown by the coefficient of variation of ± 0·3 per cent. The advantage of the simple but efficient means of stabilizing the temperature can be seen from the fact that with the apparatus constructed the free air volume was 90·4 ml., and this would involve an error of 0·99 per cent Zn for every 1 °C. change in temperature during a determination. With this free air volume a pressure variation of 1 mm. Hg would involve an error of 0·40 per cent Zn. The above errors are based on an assumed sample weight of 0·1 g.

In actual use no temperature or pressure variations were observed during the course of any individual determination, thus obviating any need for corrections (atmospheric pressure was measured to ± 1 mm. Hg and temperatures were measured to ± 0·2 °C.)

ACKNOWLEDGMENT.—*The author is indebted to the English Electric Co., Ltd., for permission to publish this paper.*

Photobiology Group

The next meeting of the Photobiology Group will be held in the Technical Optics Section at Imperial College, during the afternoon of September 29, 1961.

The programme will include papers on colour measurement, colour discrimination and colour contrast given by members of the Section, and there will be demonstrations of instruments for the measurement of colour.

Details of the programme may be obtained from:
The Secretary,
Dr. D. Vince,
Horticultural Research Laboratories,
Shinfield Grange,
Shinfield,
Berks.

THERMOGRAVIMETRIC ANALYSIS

by G. M. Lukaszewski, Ph.D., and J. P. Redfern, Ph.D., A.R.I.C.

Battersea College of Technology

PART III—TECHNIQUES USED IN CONJUNCTION WITH THERMOGRAVIMETRY

IN Part II a large number of applications of thermogravimetric analysis were briefly reviewed. However, the method on its own is not sufficiently versatile to resolve some of the problems encountered relating to a given compound or reaction and its scope can be greatly extended when it is used in conjunction with other physical techniques which are applicable to solid state chemistry. Such techniques include the following: (i) Differential thermal analysis; (ii) Hydrothermal studies; (iii) X-ray and electron diffraction studies; (iv) Infra-red spectroscopy; (v) Magnetic measurements.

Differential Thermal Analysis

This technique will be discussed first due to its close relationship to thermogravimetric analysis. The method has already achieved wide use in the study of solid transformations—especially those involving minerals and organic materials. A combination of thermogravimetric analysis and differential thermal analysis (D.T.A.) is very powerful in the provision of information concerning solid state chemical reactions especially where part of the material involved is volatile. D.T.A. involves the measurement of temperature changes which result from endo- or exothermic changes taking place in a given material in the course of heating. The temperature in the sample is recorded by means of a differential thermocouple arrangement (Fig. 10) in which one thermocouple is embedded in an inert sample while the second is embedded in a sample of the material under test. Both samples are heated simultaneously at a constant rate of rise of temperature in a single furnace. The reference material must be inert over the whole of the temperature range that is being studied.

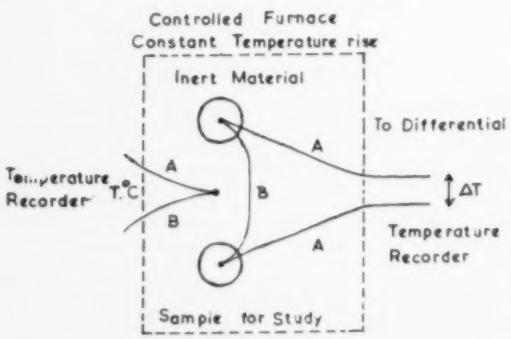

Fig. 10. Arrangement for D.T.A.

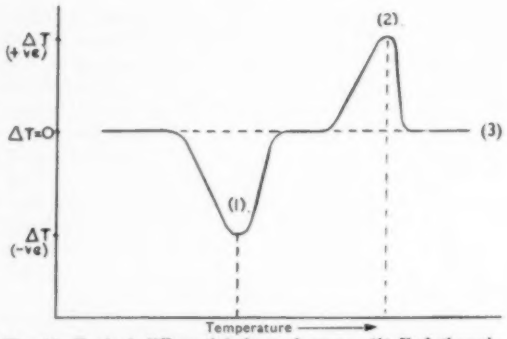

Fig. 11. Typical differential thermal curve. (1) Endothermic process [△T(—ve)]. (2) Exothermic process [△T (+ve)] (3) Reference line [△T=O].

When a phase change or decomposition reaction involving generation or absorption of heat occurs in the sample, the temperature gradient with respect to the reference material will alter. This change will be recorded as a differential e.m.f. deviating from the original value considered as an arbitrary zero (Fig. 11). The direction of the deviation relative to the zero line, when referred to the temperature gradient between the two materials, will indicate the type of heat change taking place. For example the loss of water is generally indicated by an endothermic process (1) whereas the formation of a more stable crystalline phase is indicated by an exothermic reaction (2). Simultaneous thermogravimetric analysis will help to identify the process occurring (Fig. 12).

This method easily yields qualitative information, but considerable care is needed if reliable quantitative results are to be obtained. A number of factors are important in differential thermal analysis: (*a*) the heating rate, (*b*) the sample size, (*c*) particle size, (*d*) sample holder, and (*e*) nature of the inert standard.

(*a*) *The heating rate.*—The rate of heating exerts a profound effect on the differential peaks obtained in that both the form and position of the peaks are considerably modified. An increase in the heating rate results in a shift of endothermic peaks to higher temperatures. Hence slow linear rates of heating should always be used. It is desirable that the thermogravimetric work should be carried out using a similar rate of heating in order that results are strictly comparable.

(*b*) *The sample size.*—The size of the sample has little effect on the position of the peak but exerts a considerable effect upon its shape and height whilst not materially altering the area under the curve. An

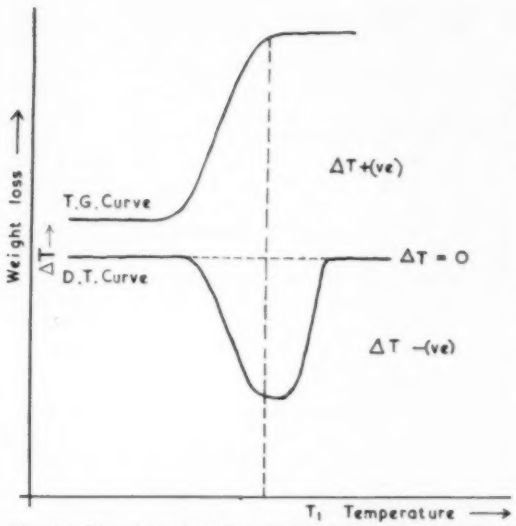

Fig. 12. Thermogravimetric and differential thermal curves showing the weight loss associated with an endothermic process.

increase in the sample size results in an increase in sensitivity. This may not, however, be conducive to good resolution in the case of two or more consecutive reactions (Fig. 13). Pask and Warner (1954) have shown that oxidation reactions are more sensitive to changes in sample size than normal decomposition reactions. The effective volume of the inert material and the sample should be equal otherwise non-uniformity in heating occurs and there will be deviations of the curves from the true shape. Certain thermocouples, e.g. Pt/Rh have a lower sensitivity than others, e.g. chromel/alumel and it is possible to compensate for this by an increase of the sample size.

(*c*) *Particle size.*—Berkelhamer (1944) observed that the shape of the differential curve is related to the particle size of the material, i.e. the extent of grinding, calcination, or ageing. This has already been discussed in the previous article in relation to thermogravimetric analysis. The effect of packing of the material is important especially around the thermocouples.

(*d*) *Sample holder.*—Sample holders should be thin-walled, of high thermal conductivity, of low heat capacity, and of small mass for the best results. Since it is very important that both the standard and the sample are uniformly heated it is necessary for the furnace to have a uniform temperature zone which extends so as to include the sample and standard. Zakharov (1939) has shown that this is best provided by containing the sample holders in a block of refractory material.

(*e*) *Nature of the inert material used as standard.*—Zakharov has indicated that so long as the standard reference material does not undergo any chemical or physical changes over the temperature range employed the nature of the standard has little appreciable effect upon the differential thermal analysis so long as it approximates the particle size and heat conductivity of the sample. Calcined alumina may conveniently be used over the range ambient temperature to 1,000°.

In order to make quantitative use of thermochemical data provided by a differential thermal analysis curve obtained with due regard to the factors outlined above it is desirable to carry out calibration experiments, for example with $CuSO_4.5H_2O$ diluted with alumina, before any attempt is made to evaluate heats of transformations. It is not the purpose of this article to review work in this field. However the reviews of Grim (1951), Smothers and Chiang (1958), and the bibliography on the subject by Smothers, Chiang, and Wilson (1951) cover the various aspects of the subject.

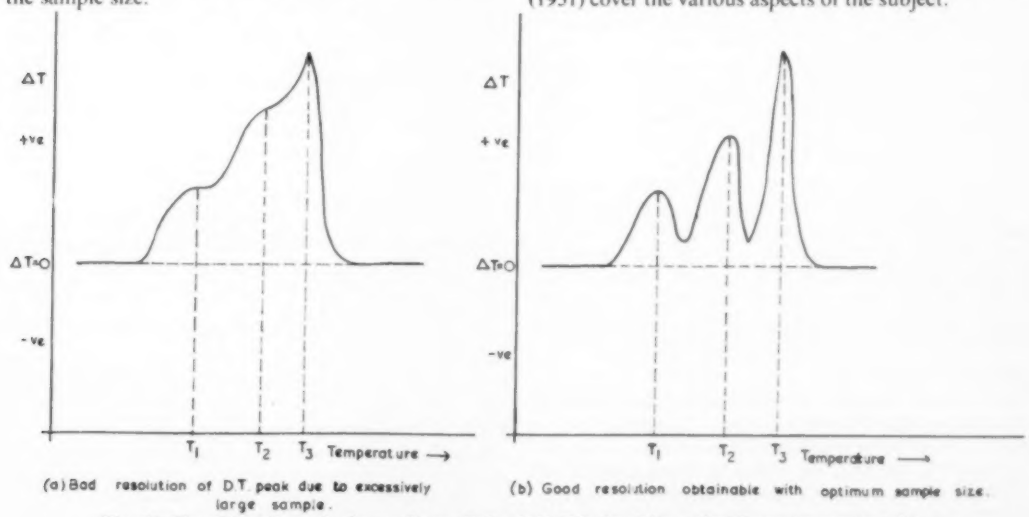

Fig. 13. The effect of sample size on the resolution in D.T.A. involving a slow linear temperature rise.

Hydrothermal Studies

As was stated in the first article some isobaric dehydrations of chemical compounds and minerals show a characteristic asymptotic curve with temperature. This may be resolved by means of the 'quasi-static' technique to give a decomposition which becomes asymptotic with time. Decomposition curves of such materials do not reveal any sharp breaks or plateaux (as do compounds which form well-defined crystalline phases) but indicate gradual transitions. Garner (1955) believes that this is typical of conditions where amorphous phases exist some time before re-crystallization. Such behaviour (which will be discussed in the final article) makes the identification of the process occurring difficult. Roy and Osborn (1952) have shown that it is often advantageous to subject such materials to treatment with varying high pressures of water whilst heating in an autoclave. In this way Shafer and Roy (1956) were able to initiate the formation of some more readily characterizable phases due to the rehydration of the amorphous phase which had been obtained by partial or complete thermal degradation. Such studies may prove of value in the interpretation of certain thermogravimetric results. Ellis and Fyfe (1957) have given a comprehensive review of the subject.

X-ray and Electron Diffraction Studies

In order to gain as much information as possible about the intermediate products obtained in thermogravimetric analysis, as indicated by plateaux or inflections in the weight-loss against temperature curve, it is useful to apply methods which yield information relating to the structure of compounds. Thus a number of studies using the thermobalance have incorporated both x-ray and electron diffraction studies. Stumpf (1950) and Rasonskaya (1956) have shown the existence of new compounds obtained by thermal degradation and verified this by x-ray examination. As the products from a thermogravimetric run are often microcrystalline or even amorphous it is impossible to carry out complete structural x-ray analysis. Nevertheless valuable information can still be gained by x-ray powder diffraction studies. Shafer and Roy (1956) have used this technique in characterizing a new phase in the system Cr_2O_3-P_2O_5-H_2O as a result of hydrothermal treatment of amorphous thermal degradation products obtained from $[Cr(H_2O)_6]PO_4$. Duval (1953) has used x-ray studies in certain of the thermogravimetric investigations of analytical methods. The application of the powder method may be extended to studies of the nature of transitions from crystalline to amorphous states as well as investigations of order-disorder transformations in the solid state as a whole.

Infra-red Spectroscopy

This technique can also be applied in a manner similar to the methods outlined in the previous paragraph. In the study of the degradation of an inorganic complex the evidence obtained by infra-red measurements will often confirm the presence or absence of certain ligands and thus the mode of the decomposition may be better understood. Infra-red studies are particularly useful in the study of the nature and behaviour of ligands in inorganic complex compounds. Thermally initiated changes may be studied with respect to loss of ligand and entry of anions into the co-ordination sphere. Such investigations can yield valuable information concerning the behaviour of the ligand, i.e. whether it is monodentate, chelating or bridging. Fujita and co-workers (1958) have discussed the application of infra-red measurements to certain inorganic complexes. Blair, Pantony, and Minkoff (1958) have studied some complexes of 8-hydroxyquinoline with vanadium in which they have combined thermogravimetric methods with infra-red and analytical data in order to characterize some of the complexes.

Magnetic Measurements

It is sometimes possible to study thermal transformations of some transition metal compounds magnetically. It is known that many compounds do not obey the Curie Law but the Curie-Weiss relationship over a range of temperatures.

$$\chi = \frac{C}{T + \theta}$$

where χ is the magnetic susceptibility, C is a constant, T and θ are in °K. θ is known as the Weiss constant, its meaning has been widely discussed (see e.g. Lewis and Wilkins, (1960)). It may be considered as a measure of the departure of the system from paramagnetic ideality. Some systems show a large value for θ and this has been explained on the basis of a magnetic interaction (antiferromagnetic in nature). Such behaviour is observed in chromium and ferric hydroxides as has been reported by Earnshaw and Lewis (1958) and previous workers. The value of θ provides certain suggestions as to the nature of the ligand group bridging between two metal atoms. In this way transformations of some polymeric compounds may be studied.

In the foregoing discussion it has been amply illustrated that thermogravimetric analysis is best used, especially in studies of inorganic or organic compounds, in conjunction with other physical methods.

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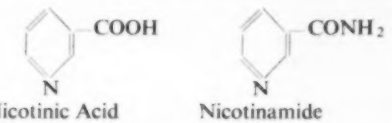
PRACTICAL METHODS FOR THE MICROBIOLOGICAL ASSAY OF THE VITAMIN B-COMPLEX AND AMINO ACIDS

by E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol.

PART II

Nicotinic Acid (Niacin) and Nicotinamide (Niacinamide)



Nicotinic Acid Nicotinamide

Organism. LACTOBACILLUS PLANTARUM

Specificity and Vitamin Requirements of *Lactobacillus plantarum*

Nicotinic acid or its amide is a specific growth factor for *L. plantarum*. This organism also responds to nicotinuric acid. Thus either the acid itself or its amide may be used for establishing a standard curve. In the early days of microbiological assay commercially produced nicotinic acid was often contaminated with appreciable amounts of inactive isomers, and a guarantee of quality of any sample used for establishing standard curves should be obtained from the manufacturers.

L. plantarum is among the less exacting of the different strains of lactic organisms used for microbiological assay work in its vitamin requirements. Three vitamins only are essential for normal growth, namely nicotinic acid or its amide, pantothenic acid and biotin; some strains require *p*-aminobenzoic acid as well. For example, thiamine, riboflavin and pyridoxine may be omitted from the basal medium described below without obvious harmful effects. On the other hand, it is undesirable on general grounds to omit them, especially as it has been claimed that they stimulate the early stages of growth.

Basal Medium

The basal medium described below is a slight modification of that originally described by Krehl *et al.* (1943) and Barton-Wright (1944).

Vitamin-free casein hydrolysate 1·0 per cent, DL-tryptophan 0·02 per cent, L-cystine 0·02 per cent, glucose 2·0 per cent, sodium acetate 2·0 per cent, xylose 0·1 per cent, adenine, guanine, uracil and xanthine 10 p.p.m. each, thiamine 1·0 p.p.m., riboflavin 0·5 p.p.m., pyridoxine 1·0 p.p.m., calcium *d*-pantothenate 1·0 p.p.m., *p*-aminobenzoic acid 0·1 p.p.m., biotin 0·001 p.p.m., sodium chloride 0·5 per cent, ammonium sulphate 0·3 per cent, potassium dihydrogen phosphate 0·06 per cent, dipotassium hydrogen phosphate 0·06 per cent and inorganic salt solution.

Basal media for assays with lactic organisms are always made up at double strength. The final volume of liquid in each tube is 10 ml. and therefore if a total volume of 1,000 ml. is required, the basal medium is made up to 500 ml. and 5 ml. aliquots added to each tube. Test tubes, 18-20 × 160-180 mm., are the most suitable size for carrying out microbiological assays.

Nicotinic Acid-free Basal Medium
(Sufficient for 100 tubes)

Vitamin-free casein hydrolysate*	10·0 g.
DL-Tryptophan solution*	50·0 ml.
L-Cystine solution*	50·0 ml.
Glucose	20·0 g.
Sodium acetate (hydrated)	33·0 g.
Xylose	1·0 g.
Adenine + guanine + uracil solution* ...	10·0 ml.
Xanthine solution*	10·0 ml.
KH_2PO_4	0·6 g.
K_2HPO_4	0·6 g.
Sodium chloride	5·0 g.
Ammonium sulphate	3·0 g.
Thiamine*	1,000 μg.
Ca-*d*-pantothenate*	1,000 μg.
Pyridoxine*	1,000 μg.
Riboflavin*	500 μg.
p-Aminobenzoic acid*	100 μg.
Biotin*	1·0 μg.
Inorganic salt solution*	5·0 ml.
Water to	500 ml.

*For the preparation of these reagents see Part III

After mixing, the pH of the medium is adjusted to 6·8 with sodium hydroxide solution using bromothymol blue as external indicator, and the volume is then made up to 500 ml. with water.

Assay Procedure

A separate standard curve must be established for every assay. The range of nicotinic acid recommended for establishing such a curve is: Blank, 0·025 μg., 0·05 μg., 0·10 μg., 0·15 μg., and 0·2 μg. A standard solution of nicotinic acid containing 0·05 μg./ml. is prepared and the following amounts plus water are added to each tube to establish such a range:

Blank, 5·0 ml. water
0·5 ml. standard solution + 4·5 ml. water
1·0 ml. standard solution + 4·0 ml. water
2·0 ml. standard solution + 3·0 ml. water
3·0 ml. standard solution + 2·0 ml. water
4·0 ml. standard solution + 1·0 ml. water.

Water and basal medium are added to each tube by

means of a calibrated B-D Cornwall Luer-Lok Syringe and standard and test solutions with an accurately standardized pipette. The assay of test samples should be carried out at at least three different concentration levels. All concentration levels of standard and test solutions are set up in duplicate if response is to be determined acidimetrically, or in triplicate if response is to be estimated nephelometrically. After the volume in each tube has been adjusted to 10 ml. with water, the tubes are capped with aluminium thimbles and sterilized. It was formerly recommended that the tubes should be autoclaved at 10 p.s.i. for 10 minutes, but it has been found by experience that the minimum amount of sterilization compatible with safety gives the best results. It is the author's practice with all assays using lactic organisms to take the tubes up to 10 p.s.i. and then start to release the pressure gradually. The temperature of incubation will depend on whether response is to be determined acidimetrically or nephelometrically. If response is to be measured by titration with 0·1 N. NaOH, the tubes are incubated at 30°C. for 72 hours, but they should be incubated at 35-37°C. for 24 hours if response is to be determined nephelometrically.

Preparation of Inoculum

It has always been the practice to prepare the inoculum for microbiological assays with lactic organisms by making a transfer from a stab culture to the full broth medium (described in col. 2), incubating for 24 hours, centrifuging aseptically, pouring off the supernatant liquid and then making a suspension of the deposit with 0·9 per cent sterile saline solution. This procedure is unnecessarily complicated and time-consuming. The author now grows the organism on a special inoculum medium which has the following composition for the assay of nicotinic acid, pantothenic acid and biotin:

Vitamin-free casein hydrolysate*	1·0 g.
Glucose	2·0 g.
Sodium acetate (hydrated)	3·3 g.
Sodium chloride	0·5 g.
Ammonium sulphate	0·3 g.
K_2HPO_4	0·06 g.
KH_2PO_4	0·06 g.
L-Cystine solution*	5·0 ml.
DL-Tryptophan solution*	5·0 ml.
Xylose	0·1 g.
Adenine + guanine + uracil solution* ...	1·0 ml.
Xanthine solution*	1·0 ml.
Inorganic salt solution*	0·5 ml.
Thiamine*	100 μg.
Riboflavin*	50 μg.
Pyridoxine*	100 μg.
p-Aminobenzoic acid*	10 μg.
Ca-*d*-pantothenate*	1·0 μg.
Nicotinic acid*	2·0 μg.
Biotin*	0·004 μg.
Water to	100 ml.

*For the preparation of these solutions see Part III

The pH is adjusted to 6·8 with sodium hydroxide solution and 10 ml. aliquots are transferred to one ounce screw-capped bottles and sterilized at 10 p.s.i. for 10 minutes. This medium will retain its activity for one month if stored in a refrigerator (4°C.) and should be discarded after this time.

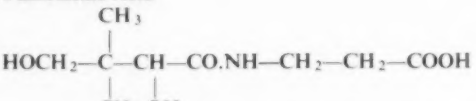
To prepare the inoculum for the assay of nicotinic acid, a transfer is made from a stab culture to a bottle of the inoculum medium and incubated for 18-20 hours at 30°C. There should be heavy growth at the end of this time. The culture is diluted 1 in 100 with quarter-strength sterile Ringer solution and one drop of the dilute suspension added to each of the assay tubes by means of a sterile pipette.

Preparation of Samples for Assay

Dry materials should be finely ground and 5·0 g. suspended in 50 ml. N.HCl and autoclaved for 15-20 minutes at 15 p.s.i. Materials containing a high percentage of fat, e.g. wheat germ, maize, oats, soybean, meat etc., should be defatted by a preliminary extraction for 16-18 hours with Analar light petroleum (B.P. 40-60°C.). This procedure prevents the formation of oily emulsions which may hinder extraction.

After autoclaving, the extract is cooled, 2·0 ml. of 2·5 M. sodium acetate solution added and the pH adjusted to 4·5 with sodium hydroxide solution using bromocresol green as external indicator, and the solution made up to volume. The solution is filtered, the pH of an aliquot readjusted to pH 6·8 with sodium hydroxide solution using bromothymol blue as external indicator, and the solution made up to its final volume. The concentration of the final solution of the extract should be so adjusted as to contain as nearly as possible 0·04-0·05 μg./ml. of nicotinic acid. Although 5·0 g. of material is recommended for extraction, smaller amounts should be used for high potency material, such as dried yeast (nicotinic acid content up to 600 μg./g.) and wheat bran (nicotinic acid content up to 325 μg./g.). In such cases 1·0 g. samples will suffice.

Pantothenic Acid



Pantothenic acid

(N-(αγ-dihydroxy-β-dimethyl-butyryl)β-alanine)

Organism. Lactobacillus plantarum

Basal Medium

The author has found the basal medium described below to give very satisfactory results for the assay of pantothenic acid.

Sodium hydroxide-treated peptone 0·5 per cent, vitamin-free casein hydrolysate 0·2 per cent, L-cystine 0·02 per cent, DL-tryptophan 0·02 per cent, glucose 2·0 per cent, sodium acetate 2·0 per cent, xylose 0·1

per cent, adenine, guanine, uracil and xanthine 10 p.p.m. each, nicotinic acid 2·0 p.p.m., riboflavin 0·5 p.p.m., pyridoxine 2·0 p.p.m., *p*-aminobenzoic acid 0·1 p.p.m., biotin 0·001 p.p.m., sodium chloride 0·5 per cent, ammonium sulphate 0·3 per cent, dipotassium hydrogen phosphate 0·06 per cent, potassium dihydrogen phosphate 0·06 per cent, and inorganic salt solution.

Pantothenic Acid-free Basal Medium
(Sufficient for 100 tubes)

Sodium hydroxide-treated peptone + sodium acetate*	100 ml.
Vitamin-free casein hydrolysate*	2·0 g.
L-Cystine solution*	50·0 ml.
DL-Tryptophan solution*	50·0 ml.
Glucose	20·0 g.
Sodium acetate (hydrated)	22·0 g.
Xylose	1·0 g.
Adenine + guanine + uracil solution* ...	10·0 ml.
Xanthine solution*	10·0 ml.
Sodium chloride	5·0 g.
Ammonium sulphate	3·0 g.
K_2HPO_4	0·6 g.
KH_2PO_4	0·6 g.
Thiamine*	1,000 µg.
Pyridoxine*	2,000 µg.
Riboflavin*	500 µg.
Nicotinic acid*	2,000 µg.
p-Aminobenzoic acid*	100 µg.
Biotin*	1·0 µg.
Inorganic salt solution*	5·0 ml.
Water to	500 ml.

*For the preparation of these reagents see Part III

After mixing the pH of the medium is adjusted to 6·8 with sodium hydroxide solution using bromothymol blue as external indicator, and the volume made up to 500 ml. with water.

Assay Procedure

The assay is carried out in exactly the same way as for nicotinic acid, i.e. 5 ml. portions of basal medium are transferred to test tubes, the standard calcium *d*-pantothenate and test solutions added and the total volume of liquid in the tubes is made up to 10 ml. with water. The range of calcium *d*-pantothenate recommended for establishing a standard curve, which must be prepared afresh for each new set of assays, is: Blank, 0·02 µg., 0·04 µg., 0·06 µg., 0·08 µg., and 0·1 µg. A standard solution of calcium *d*-pantothenate is prepared containing 0·02 µg./ml. of the salt and the following amounts plus water added to each tube to establish such a curve:

Blank, 5·0 ml. water
1·0 ml. standard solution + 4·0 ml. water
2·0 ml. standard solution + 3·0 ml. water
3·0 ml. standard solution + 2·0 ml. water
4·0 ml. standard solution + 1·0 ml. water
5·0 ml. standard solution.

The assay of each test sample is carried out at three or four different concentration levels. As in the assay of nicotinic acid, all concentration levels of standard and test solutions are set up in duplicate if response is to be estimated acidimetrically and in triplicate if response is to be determined nephelometrically. After the volume in each tube has been adjusted to 10 ml. with water, the tubes are capped with aluminium thimbles and sterilized in the manner described for the assay of nicotinic acid. The temperature of incubation will depend upon whether response is determined acidimetrically, i.e. titration with 0·1 N. NaOH (30 °C. for 72 hours) or nephelometrically (35-37 °C. for 24 hours). The inoculum is prepared in the same way as for the assay of nicotinic acid.

Preparation of Samples for Assay

Pantothenic acid is unstable towards alkalis and strong acids, and extraction must therefore be carried out enzymatically. The enzymic extraction of pantothenic acid from its combined form is difficult and many of the methods suggested in the past have proved to be unsatisfactory. As far as the author is aware, the most satisfactory method known at present is that put forward by Neilands and Strong (1948) who recommended that hydrolysis should be carried out using a mixture of an alkaline phosphatase and an enzyme prepared from chicken or pigeon liver.

The chicken or pigeon liver enzyme is prepared as follows:

The livers are removed from freshly killed birds, chilled to approximately 4 °C., minced and homogenized with 20 volumes of cold acetone in a Waring blender. The precipitate is washed with acetone and ether. The enzyme is now extracted from the precipitate by washing with 10 times its weight of ice-cold 0·02 M. sodium bicarbonate solution and removing solids on the centrifuge. The enzyme in solution is labile at room temperatures and should be maintained frozen in small quantities.

The preparation of the alkaline phosphatase from calf liver by the method of Schmidt and Thannhauser (1943) is very difficult under ordinary laboratory conditions and more often than not an inactive product is obtained. This enzyme as well as the dried pigeon liver may now be purchased from either L. Light & Co., Colnbrook, Bucks. or the Worthington Biochemical Corporation (U.K. agents V. A. Howe & Co., 46 Pembridge Road, London, W.11.)

In order to achieve maximum liberation of pantothenic acid a minimum amount of substrate should be used, thus keeping the concentration of enzyme in excess. The incubation period is short, 4 hours at 37 °C. The enzymes must be assayed for pantothenic acid and the amount deducted from the test assay value. (See in this connection Clegg (1958)).

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(*To be continued*)

VARIATIONS AND ERRORS IN EXPERIMENTAL INVESTIGATIONS

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PART VI—PROCEDURES, ARTEFACTS AND PITFALLS

ONE of the factors which concern an investigator is the possibility that the experimental procedure or the theoretical edifice used may prove to be an artefact* or an illusion which will lead to a pitfall.

Artefacts are believed to have been encountered in a number of biological fields. Thus, spindle formations which can be readily demonstrated in stained preparations of cells undergoing mitosis or meiosis have been considered by a number of biologists to be artefacts which arise from the actual process of staining. Another case is concerned with the physical classification of human sperms into the X- and Y- categories according to size. Shettles (1960) interpreted photographs of sperms taken in his laboratory as showing distinct differences in size and shape and which support the above classification. Lord Rothschild (1960), however, considered these to be artefacts.

In the biological sense artefacts are structural illusions as they have been caused by 'faulty' preparation caused by man. If scientific licence be allowed, the term may be extended to include optical illusions or any other scientific structural illusions including erroneous theoretical edifices. Many such illusory edifices have existed in scientific fields in the past, and probably several still exist, some unrecognized. As artefacts may well lead to erroneous conclusions followed by pitfalls, it is desirable to recognize them as such. However artefacts, and illusory edifices in general, should not be condemned out of hand. There is a number of cases in which the theoretical structure or assumptions have been erroneous, but where the conclusions based on them have nevertheless been found to be correct, as evidenced by other considerations. For example, Boyle's Law is considered to be more accurate as the pressure becomes progressively smaller. Hence it should apply exactly at zero pressure. Indeed, extrapolation of the density of the gas to zero pressure yields a value which is used for computing its accurate molecular weight, and this value agrees with that determined by independent methods. Yet at pressure values approaching zero the number of collisions of the gas molecules per sq. cm. per second with the walls of the containing vessel becomes so small as to cause misgivings as to its statistical meaning. For example, as many as 10 seconds may elapse before a collision is experienced by the wall of the vessel, and during this time pressure is not experienced. Another example is to be found in the use of Charles' Law for the assessment of the absolute zero of temperature (Lewin, 1961).

Returning to the consideration of experimental techniques when they are responsible for erroneous conclusions or for lack of reproducibility one finds that usually sufficient care is exercised to avoid faulty techniques and wrong sequence of operations. However one sometimes encounters lack of appreciation that simple steps, such as those of the dissolving of a substance, can be the source of error or lack of reproducibility.

Consider for example the preparation of a concentrated solution of glucose. This can be readily prepared when the finely divided solid is slowly added to vigorously stirred water. The procedure ensures that each particle of the solid is surrounded by the liquid and that the association of the surface glucose molecules with the water is followed immediately by their detachment from the solid into the bulk of the surrounding liquid, owing to the vigorous agitation. Had this process not been carried out sufficiently rapidly, the solid glucose monohydrate formed initially on the surface of the solid glucose would have formed lumps which ordinarily dissolve very slowly. If the reverse procedure had been adopted, viz. had the water been added slowly to the solid glucose, the lumps formed would have required a day or two of continuous stirring for total solution to be effected. Indeed, the first impression created would have been that glucose does not dissolve in water; a 'fact', or artefact, created by the particular experimental procedure.

A parallel phenomenon is encountered when an aqueous 'solution' of starch or agar-agar is to be made. Such 'solutions' (they are really lyophilic colloidal suspensions) are readily made by slow addition of the finely divided solid to the hot water which is strongly agitated. Yet the procedure often recommended is to add small quantities of water to the starch, or agar-agar, mixing the two thoroughly to form a paste which is then heated with more water until the original paste dissolves

*The variations of the definition of the term artefact, or artifact, given by different dictionaries are so pronounced that some deserve quoting:
Chambers' Twentieth Century Dictionary (New ed., 1959) gives: 'A thing made by human workmanship'.
Chambers' Technical Dictionary (1958) states: 'A man-made implement: (Zool.) An apparent structure which does not represent part of the actual specimen, but is due to faulty preparation'.
Dictionary of Difficult Words (R. H. Hill, 1958) gives: 'Object of human manufacture; Condition caused by human interference'.
Faber Medical Dictionary (1953) states: 'An artificial product resulting from a physical or chemical agency'.
The Encyclopaedia Britannica (1949) states: 'An object fashioned by man'.
Examination of several other dictionaries showed parallel variations in the definition of the term.

completely. Considerably longer time is spent, in the production of an even paste and in dissolving it, than in the method advocated above.

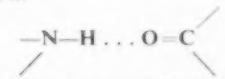
In the preparation of glucose solutions, it is probable that whatever procedure is adopted, the final glucose solutions would have had the same physical constitution and properties. In the case of starch and agar-agar it is not certain whether the properties of the differently prepared suspensions would have been the same initially, as the degrees of hydration and of particle association may have initially been different. However, after a reasonable interval of time it is to be expected that they would be practically the same. There are cases where this conclusion does not apply, and where the different sequence of the individual steps, which involve only mixing, results in the two solutions having different physical, or even chemical, properties.

Consider in this connection the chemical preparation of lyophobic suspensions by simple mixing. The major factor in determining the nature of charge on the lyophobe is whether the positive ions or the negative ions forming part of the colloid are present in excess. Thus, when colloidal silver chloride is formed, the particles are likely to possess a positive charge if the chloride containing solution is added to an excess of silver ion solution, and *vice versa*. Also, the magnitude of the charge is related to the rate of addition or mixing. The colloidal suspensions once formed display therefore fundamentally different physical properties. Further, as such colloidal suspensions are ordinarily very stable, once they are dialysed to remove excessive electrolyte material the passage of a reasonable interval of time will not assist in the attainment of physical equality of oppositely charged suspensions of the same colloidal species.

Another comparatively simple category is one in which a given preparation under one set of conditions allows correction of the effects of a certain sequence because there the effects are reversible, while under another set of conditions involving irreversibility the incorrect sequence has adverse results. This is to be found in preparations of solutions capable of being oxidized by air, e.g. the preparation of a stable ferrous sulphate solution. Ordinarily, one acidifies the distilled water *before* addition of the solid ferrous sulphate. This stops the hydrolysis of ferrous sulphate to ferric hydroxide and the oxidation of ferrous ion to ferric by the dissolved oxygen. It is unsatisfactory to acidify the prepared solution because in the meantime some oxidation is likely to have taken place. On the other hand, under anaerobic conditions oxidation does not take place, and subsequent acidification is satisfactory in that it can reverse any hydrolysis which may have taken place.

Irreversible changes may take place on solution in other cases. This applies in the process of solution of deoxyribonucleic acid (DNA) and similar compounds which possess hydrogen bonds. The case of DNA is interesting not only because it is a most important macromolecule, but also because of the hidden pitfalls facing seasoned investigators attacking new fields. The lack of reproducibility of results in different laboratories was attributable originally to different methods of preparation. However, even when identical sources and extraction techniques have been used, variations in the properties of the material were still noted. One source of error could be the small variations in time intervals during which the extraction of DNA required the use of *high* speed mixers and the use of strong detergents or phenol which is capable of adversely affecting the hydrogen bonds in the DNA. These factors are now recognized and efforts are made in the various laboratories to decrease as far as possible their effects.

The preparation of a solution of DNA in water proved eventually to be a source of lack of reproducibility not only between different laboratories, but even in the same laboratory. This has been shown by the use of the various techniques and methods with which the properties of DNA are being evaluated, e.g. viscosity, spectrophotometry, temperature effect, sedimentation and electrometric measurements. It was found that at low electrolyte concentration some of the DNA became denatured, e.g. some of the hydrogen bonds involved in



were broken up. The degree of denaturation is dependent on the electrolyte dilution and on the age of the solution. It is nowadays accepted that DNA solutions should not be made in distilled water but rather in electrolyte solutions, e.g. NaCl solutions the concentration of which should be not below 0·001 M; smaller concentrations are allowable when divalent cations are used. (Butler, Laurence, Robins and Shooter, 1959.)

The formation of non-stable states (Lewin, 1953, 1960) may be considered as physical or chemical illusions, so far as stability is concerned, and which may well result in pitfalls. Many illustrations are available in this field, but only few will be given:

(*a*) *Non-stable emulsions*. The vigorous shaking together of two immiscible solvents, e.g. a small quantity of toluene with a large quantity of water, results in a very fine emulsion which is undetectable by eye and which can be stable for several minutes.

(*b*) *Solubility*. In attempting to dissolve a given substance it is occasionally ground very finely. However, the solubility of very fine particles is greater than that of large ones. Further, insoluble substances when ground in colloidal mills and then shaken vigorously with water form very fine colloidal suspensions which are undetectable by the unaided eye.

(*c*) *Rapid crystallization and rapid precipitation*. Rapid crystallization usually gives rise to small crystals. A double pitfall exists here in that the solubility values obtained are bound to be larger than those referring to true equilibrium and in that when the crystals take a long time to change to larger ones, a metastable form

sometimes produced may be erroneously believed to be the stable form. Metastable solid formation is indeed known fairly widely. Thus, metastable forms have been noted in the preparation of hydrated calcium tartrate (Chattaway, 1916) and of 2:4 dibromo-acetanilide (Chattaway and Lambert, 1915), and of course many metastable forms are found in the precipitations observed in qualitative analysis.

The existence of artefacts in paper electrophoresis when more than one spot may be obtained for a given substance when overheating takes place are too well known to need description. However, mention should be made of the pitfalls caused by the tendency of some substances to breakdown under high potential gradients, thus giving rise to artefacts if taken as demonstrating the presence of two compounds in the original solution where only one existed.

In the field of precipitation of agar-gel immunodiffusion plates quite a number of pitfalls may be recognized (Feinberg, 1957a, 1957b, 1960).

A number of possible pitfalls have been recognized in paper chromatography where multiple spot formation for a single substance has often been encountered. For example, Beckett, Beavan and Robinson (1960) found that the acid salts of certain pharmacologically active pure amines, e.g. adrenaline, codeine, and amphetamine, gave two spots on paper chromatograms. The phenomenon is readily demonstrable when the organic acid of the salt has a low pKa value and a high R_F value (e.g. trichloracetic acid pKa = 0·65, R_F = 0·90), and the amine and the acid have different R_F values. Of the two spots which give staining reactions for the amine, one corresponds to that of the amine itself, the other with that of the organic acid. The phenomenon is explainable in terms of association of the organic acid with the amine which is sufficiently strong to overcome in part the breaking up tendency due to the selective adsorptions and desorptions of the individual constituents.

We have seen that pitfalls can be encountered in scientific investigations in 'simple' as well as more complicated operations. It is precisely because artefacts, illusions and pitfalls can be very prevalent that one of the first principles in scientific work is to take as little as possible for granted. This applies particularly in completely new fields where the possibility of misinterpretations is far greater than in fields which have been under examination for some time.

The risk of falling into pitfalls can be effectively decreased by the use of many types of 'blank' or control experiments. These may appear sometimes as unnecessary and tedious, and may eventually have been proved to be superfluous. Nevertheless, experience has repeatedly shown that rushing through an investigation enhances the probability of error and therefore increases the amount of work rather than decreases it. Indeed, despite careful work many investigators prefer to defer publication of the work for some time so as to enable a later reconsideration, thus decreasing the probability of misinterpretation.

This series of articles on the subject of variations and errors in experimental investigations, now concluded, is not meant to be indicative of all the various possible sources of errors. It is intended merely as a means of drawing attention to some of these in the hope that it will initiate a chain of thought that will tend to examine critically any evidence capable of being misinterpreted, and to put the young investigator on his guard against methods or procedures which increase the probability of falling into pitfalls.

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Recent British Standards

Chemical Analysis of Butter

This revised British Standard (B.S. 769:1961, price 7s. 6d.) supersedes the 1952 edition and includes modifications to a number of the standard methods of analysis as well as a new clause relating to the acidity of butterfat.

The standard lays down methods for the preparation of the sample and includes methods for determining moisture, curd and salt, salt, curd, fat, titratable acidity, volatile acids, iodine value, saponification value, refractive index of butterfat, copper, iron, and pH of butter serum.

Reference Tables for Thermocouples

The British Standards Institution has published another set of reference tables for the purpose of converting thermocouple voltages into equivalent temperatures. The new tables are published as B.S. 1828: Reference tables for thermocouples (copper *v.* constantan) (price 6s.).

Like the other reference tables in this series (B.S. 1826 and 1827), they are based on the International Temperature Scale 1948 and on the Absolute Volt which in 1948, was adopted as the standard unit in place of the International Volt.

The electromotive force/temperature curve adopted as a basis for the tables is one typical of the wire supplied by British manufacturers, since, after extensive research, it has been found impracticable for British wire manufacturers to produce copper-nickel alloy in bulk which will give values in accord with the tables of the United States National Bureau of Standards.

Copies of these standards may be obtained from the British Standards Institution, Sales Branch, 2 Park Street, London, W.1.

OCTOBER ISSUE

A further series of Exhibition Lectures will be featured in the October issue of LABORATORY PRACTICE.

METHODS FOR THE EVALUATION OF THE ANTI-BACTERIAL ACTIVITY OF SURFACE ACTIVE COMPOUNDS: TECHNICAL ASPECTS OF THE PROBLEM

by J. G. Davis

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PART II

4. Factors affecting Tests for Antibacterial Activity

It is now generally agreed that the only completely satisfactory way to test germicidal preparations is to use them under the actual conditions in which they will be used in practice. Even then the method of assessment and the end point must be chosen arbitrarily; or alternatively their efficiency may be evaluated in terms of prevention of infection or of a satisfactory keeping quality for a food. However, laboratory tests can be very useful for 'sorting' purposes provided they are properly designed. The nearer the laboratory test conditions are to the conditions of use the more reliable will the results be. Practical conditions can never be standardized because they vary from day to day; hence laboratory tests should be regarded as giving information complementary to that obtained by practical use tests.

The following factors may affect the result of any test on a disinfectant.

(a) *The test organism*

(i) *Choice*. Apart from the few well-known exceptions, infections and food spoilage are caused by non-sporulating, non-acid-fast organisms which are relatively susceptible to disinfectants and antiseptics.

Few organic compounds are effective against all types of micro-organism, but surface active germicides especially have a restricted 'spectrum' of activity, often being highly toxic to some bacteria and relatively innocuous to others. It follows that the choice of test organism is of great importance. The one chosen should be as appropriate as possible to the problem.

From the testing aspect the public health interest appears to be greater than the food spoilage interest. Most of our work has been done with the former aspect in mind, and the following representative organisms have been used. Gram-positive; *Staphylococcus aureus*, *Streptococcus pyogenes* and *Strep. faecalis*: Gram-negative; *Salmonella typhi*, *Escherichia coli* I and *Pseudomonas aeruginosa*.

In our experience all QACs are so effective against Gram-positive organisms that there is little point in trying to assess degrees of effectiveness. The Gram-negative species are almost invariably more resistant; *Salm. typhi* and *E. coli* behave similarly, but *Ps. aeruginosa* is appreciably more resistant than these and in practice any treatment by a QAC preparation which is satisfactory against the latter can be relied upon to be effective against the other five. *Strep. pyogenes* is usually the least and *Strep. faecalis* the most resistant of the Gram-positive cocci, but from the point of view of potential danger *Staph. aureus* is the most appropriate test organism. However, *Strep. faecalis* is likely to give more reproducible results as it does not form clusters as does *Staph. aureus*. If *Ps. aeruginosa* is not important *E. coli* can safely be accepted as the test organism as it is invariably more resistant than Gram-positive organisms (except spores) and is usually more resistant then *Salm. typhi*. Typical results are given in Table II. With soap-phenol mixtures Berry (1952) concluded that *E. coli* was the most suitable for weak preparations and *Ps. aeruginosa* for strong ones. The latter was actually more resistant to phenol than heat resistant spores, an illustration of the fact that germicide resistance and heat resistance are fundamentally different properties.

TABLE II

The survival of different organisms in wash liquor (dish-washing) containing a non-ionic detergent and a QAC (Davis, unpublished data)

Organism	*Colony count/ml. after (min.)*				
	0	1	2	3	4
Ps. aeruginosa	52,000	4,900	2,920	2,360	2,290
E. coli	3,100,000	42,000	40	< 10	< 10
Salm. typhi	1,180,000	600	10	< 10	< 10
Staph. aureus	870,000	< 10	< 10	< 10	< 10

Broadly speaking, the QACs are much more effective against Gram-positive bacteria than against Gram-negative bacteria or yeasts, and moulds are more resistant than yeasts. They are virtually useless against spores (Davies, 1949), a concentration of 10,000 p/m being necessary to kill those of *Bacillus subtilis* in 4 hr. (Steingold *et al.* 1954).

In chemical sterilization procedures in the public health field *Mycobacterium tuberculosis* still remains the greatest problem. Claims for powerful germicidal activity against this organism appear regularly for all types of compounds, but in our experience these claims are rarely substantiated. Together with *Ps. aeruginosa*, *M. tuberculosis* may be regarded as the test organism *par excellence* for any new germicide for which extraordinary claims are made. Claims for effectiveness against *M. tuberculosis* have recently been made for some of the ampholytic compounds; for a new member of this group one manufacturer has claimed a Rideal-Walker coefficient of 37 for a 50 per cent preparation.

(ii) *Origin and condition.* In standard methods the use of a certain culture from a recognized collection is usually specified; but such cultures may change their characteristics with age, and laboratory maintained cultures may be less resistant than freshly isolated strains from infections. We recommend, therefore, that a freshly isolated strain, preferably from an infection or faulty product, should be used as well as a type culture in disinfectant testing.

Physiological condition is usually standardized by using a 24 hr. broth or agar slope culture after three serial 24 hr. sub-cultures at 37° or other suitable temperature. The culture medium is also standardized, but materials such as peptone, even when specified by name, can never be guaranteed to be constant in behaviour. If a suspension is prepared from an agar slope culture by washing the cells off with Ringer's solution they may be weakened (cf. section 4, d vi) but this technique gives a suspension relatively free from medium constituents. Direct addition of a broth culture avoids this washing of the cells but involves the addition of a small amount of medium to the test solution.

Errors can also arise from clumping of the cells, which occurs readily with such species as *Staph. aureus*. Adsorption of a surface active germicide on such clumps may help to prevent their disintegration and so protect the inner cells from the germicide. Bean and Berry (1951) observed that soap solutions tended to clump bacteria, especially *Ps. aeruginosa*. Colony counts will also be affected by the degree of clumping.

(iii) *The initial number of cells.* Apart from any specific interaction between germicides and constituents of bacterial cells, the cells themselves behave as organic matter and so will tend to weaken disinfectant action. It is therefore necessary to standardize the concentration of cells either in the medium or suspension or on the surface of the test object.

A convenient initial number of viable cells (units forming colonies) for standardization purposes is 10^6/ml. of medium, or of rinse if surfaces are to be disinfected. This number is not large enough to introduce organic matter effects or to favour clumping, but is sufficient to provide a reasonable count for a 99·99 per cent kill. It is advisable therefore to work with an initial count in the range $0{\cdot}5—2{\cdot}0 \times 10^6$/ml. Some workers have found that reproducibility suffers if initial counts appreciably higher than 10^6/ml. are used. However, if a 99·999 per cent kill is adopted as the criterion of effectiveness, a higher initial count is necessary. Thus Ortenzio (1958) suggested an initial count of $75—125 \times 10^6$/ml.

(iv) *The nature of the recovery medium.* Of the six organisms mentioned above only *Strep. pyogenes* is particularly fastidious in its growth requirements. Selective media are available for all six cultures but they tend to be inhibitory even to the organism in question and there is no special advantage in using them in disinfectant testing. Media permitting luxuriant growth of the test organism may, however, not only give damaged cells the best opportunity to recover (see e.g. Jacobs and Harris, 1960), but by allowing contaminants to grow they automatically provide a continual check on technique.

For routine testing we use a medium containing: peptone, 5 g; Yeastrel, 5 g; glucose, 5 g; NaCl, 5 g; milk (whole), 10 ml. and tap water (known satisfactory), 1,000 ml. This solution is adjusted to pH 6·8, autoclaved momentarily, filtered, tubed and autoclaved. For solid media agar (15 g.) is included. Suitable modifications to this basic medium can be made for particular organisms. With *Strep. pyogenes* 10 per cent of serum is added at the time of plating or culturing.

All media constituents must be checked for suitability; this is normally done automatically in frequent testing, any anomalous behaviour of any organism on a new batch of medium being immediately recognized by an experienced worker. When special media constituents are prescribed for specific tests, e.g. the Rideal-Walker coefficient by the Crown Agents' method, these should be used. However, it seems unnecessarily complicated to recommend different peptones for similar tests, and simplification of all official methods in this respect is highly desirable.

(b) *The environment of the test organism*

(i) *The chemical quality of the water.* Hard waters may considerably reduce the bactericidal power of QACs (Table III).

TABLE III

The effect of the nature of the water on the bactericidal action of three QACs*

(Data from Report, 1947)

QAC	Lethal concn. (p/m) when used in			Use concn.† (p/m)
	Distilled water	Cincinnati water	Norwood water	
A	20	100	250	234
B	10	70	220	190
C	5	500	3,200	78

* Concentration giving 100 per cent kill of *E. coli* in 1 min.

† Recommended by the makers.

It was found that pH, organic matter, anions and monovalent cations had no significant effect, but 40 p/m of calcium or magnesium could reduce the kill against *Salm. typhi* from 99·9 per cent to 50 per cent. However, this hardness effect is not generally apparent with Gram-positive organisms (Johns, 1948; Lawrence, 1950, p. 36). The adverse effect of calcium salts may be removed by adding small quantities of alkaline detergents such as trisodium phosphate. Cousins and Clegg (1956) emphasized that not only do QACs vary in their behaviour in hard waters, but the effects of added detergents also vary. Nothing can be assumed.

(ii) *The effect of pH.* This can be considerable. It varies with the particular QAC but bactericidal effect usually increases with increasing pH (Dunn, 1937; Gershenfeld and Milanick, 1941; Gershenfeld

and Perlstein, 1941). Typical results are given by Lawrence (1950, p. 78). However, there are many anomalies (cf. Hucker *et al.* 1948) and the pH effect may vary according to the organism used (Salton, 1950; Soike *et al.* 1952). In the region of pH 4, QACs become relatively ineffective, but cetylpyridinium chloride appears to be an interesting exception (Quisno and Foter, 1946). It should not be forgotten that the pH of a solution may change on standing (Stuart, 1947).

It seems that the nature of the buffer may be at least as important as the pH itself (Clarke and Garvie, 1955), a point frequently overlooked in both bacteriological and biochemical work. The concentration of other ions is usually altered even if only one buffer is used, and if different buffers are used in pH range experiments at least two other variables are introduced. It is impossible to alter a solution in pH alone.

(iii) *The degree of aeration.* In a laboratory test the degree of aeration is probably fairly constant, but under practical conditions, especially where large volumes are concerned, the oxygen tension may vary and could conceivably affect germicidal power against obligate aerobes and perhaps even against facultative anaerobes.

(iv) *Temperature.* The rate of killing invariably increases with temperature, but different QACs possess widely different temperature coefficients. Thus two QACs may be significantly different in effectiveness at 20° but about equal at 40° (Cousins, 1951).

Many disinfection tests are made at 20°, though a temperature of 37° may be used, especially with antiseptics. Bearing in mind that detergent-sanitizers are invariably used at least warm we have standardized on a temperature of 45° for the reason that this is the highest temperature for the comfortable hand-washing of dishes and equipment in general.

(v) *Organic matter.* It has long been known that disinfectants are greatly weakened in the presence of organic matter (Chick and Martin, 1908). Although QACs may be less sensitive than other disinfectants (Domagk, 1935; Hornung, 1935; Kliewe and Maier, 1936) their germicidal powers are invariably weakened. One well-known illustration of this effect is the fact that the spread of Rideal-Walker coefficients is much greater than that of Chick-Martin coefficients. In other words organic matter tends to level out differences between disinfectants. Cousins (1951) observed that inconsistencies in survivor counts were reduced when milk was added to the test solutions.

A good example of how organic matter can nullify the results obtained by orthodox *in vitro* tests is afforded by the performance of mouth washes. The phenol coefficient test is virtually useless (American Dental Association, 1932). Welch and Brewer (1942) found that out of 87 proprietary mouth washes only nine possessed any appreciable germicidal power in use. Wolf and Brown (1946) found that in as short a time as 10 min. after the use of a mouth wash the mouth flora showed little change, and was back to normal after 1 hr.

It can be expected that the greater the amount of organic matter, and the more firmly it is attached to the equipment or tissue being 'sanitized,' the more important is the detergent power of any sanitizing preparation. Thus Wolf and Brown (1946) observed that of all the preparations tested, which included some very powerful *in vitro* germicides, the most effective was 1 per cent soap solution which, by *in vitro* tests, would be rated as very feeble.

It is an easy matter to incorporate a known amount of organic matter in any test. The obvious material to choose is that which is normally present under the conditions of use, e.g. serum, blood, faeces, urine, milk, or even excess of the cells of the organism to be killed. Constancy of composition is essential and in practice availability and convenience are also important factors. For standardization purposes there is much to be said for selecting one such material, and after trying nearly all the usual soiling materials we have come to the conclusion that bulk whole milk is the best. The five reasons for this decision are that it is practically constant in composition, it contains fat, protein, sugar and salts in almost constant proportions, it is universally available at all times, volumes can be rapidly measured, and it is more protective than most other organic materials which have been used for this purpose.

Much of our work has been in relation to dish washing, which is probably a typical operation from the points of view of use, type of soiling material and time of treatment. We have found that dish washing solutions contain when discarded after use about 0·5 per cent of soiling matter on a dry weight basis, and we have therefore standardized on an addition of 4 per cent of whole milk (0·5 per cent dry matter) in our disinfectant testing. Typical results are given in Tables IV and V. For antiseptics albumin is suitable and convenient and the concentration may be varied appropriately; e.g. for mouth wash tests it should correspond to the protein content of saliva.

TABLE IV

The effect of organic matter on the bactericidal powers of three non-ionic detergent QAC mixtures against Salm. typhi
(Davis, unpublished data)

Addition	Kill (%) with detergent-QAC mixture no.		
	1	2	3
None	99·998	99·975	99·985
	99·852	99·999	99·961
	99·999	99·985	99·922
Peptone (0·5%)	99·998	99·997	90·9
	99·992	99·840	99·977
	99·999	99·970	99·961
Milk (4%)	99·967	75·2	83·2
	99·995	72·2	88·1
	99·999	70·0	86·2

The effect of organic matter depends not only on its nature and concentration but on the test organism,

(ii) *Origin and condition.* In standard methods the use of a certain culture from a recognized collection is usually specified; but such cultures may change their characteristics with age, and laboratory maintained cultures may be less resistant than freshly isolated strains from infections. We recommend, therefore, that a freshly isolated strain, preferably from an infection or faulty product, should be used as well as a type culture in disinfectant testing.

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(iv) *Temperature.* The rate of killing invariably increases with temperature, but different QACs possess widely different temperature coefficients. Thus two QACs may be significantly different in effectiveness at 20° but about equal at 40° (Cousins, 1951).

Many disinfection tests are made at 20°, though a temperature of 37° may be used, especially with antiseptics. Bearing in mind that detergent-sanitizers are invariably used at least warm we have standardized on a temperature of 45° for the reason that this is the highest temperature for the comfortable hand-washing of dishes and equipment in general.

(v) *Organic matter.* It has long been known that disinfectants are greatly weakened in the presence of organic matter (Chick and Martin, 1908). Although QACs may be less sensitive than other disinfectants (Domagk, 1935; Hornung, 1935; Kliewe and Maier, 1936) their germicidal powers are invariably weakened. One well-known illustration of this effect is the fact that the spread of Rideal-Walker coefficients is much greater than that of Chick-Martin coefficients. In other words organic matter tends to level out differences between disinfectants. Cousins (1951) observed that inconsistencies in survivor counts were reduced when milk was added to the test solutions.

A good example of how organic matter can nullify the results obtained by orthodox *in vitro* tests is afforded by the performance of mouth washes. The phenol coefficient test is virtually useless (American Dental Association, 1932). Welch and Brewer (1942) found that out of 87 proprietary mouth washes only nine possessed any appreciable germicidal power in use. Wolf and Brown (1946) found that in as short a time as 10 min. after the use of a mouth wash the mouth flora showed little change, and was back to normal after 1 hr.

It can be expected that the greater the amount of organic matter, and the more firmly it is attached to the equipment or tissue being 'sanitized,' the more important is the detergent power of any sanitizing preparation. Thus Wolf and Brown (1946) observed that of all the preparations tested, which included some very powerful *in vitro* germicides, the most effective was 1 per cent soap solution which, by *in vitro* tests, would be rated as very feeble.

It is an easy matter to incorporate a known amount of organic matter in any test. The obvious material to choose is that which is normally present under the conditions of use, e.g. serum, blood, faeces, urine, milk, or even excess of the cells of the organism to be killed. Constancy of composition is essential and in practice availability and convenience are also important factors. For standardization purposes there is much to be said for selecting one such material, and after trying nearly all the usual soiling materials we have come to the conclusion that bulk whole milk is the best. The five reasons for this decision are that it is practically constant in composition, it contains fat, protein, sugar and salts in almost constant proportions, it is universally available at all times, volumes can be rapidly measured, and it is more protective than most other organic materials which have been used for this purpose.

Much of our work has been in relation to dish washing, which is probably a typical operation from the points of view of use, type of soiling material and time of treatment. We have found that dish washing solutions contain when discarded after use about 0·5 per cent of soiling matter on a dry weight basis, and we have therefore standardized on an addition of 4 per cent of whole milk (0·5 per cent dry matter) in our disinfectant testing. Typical results are given in Tables IV and V. For antiseptics albumin is suitable and convenient and the concentration may be varied appropriately; e.g. for mouth wash tests it should correspond to the protein content of saliva.

TABLE IV

The effect of organic matter on the bactericidal powers of three non-ionic detergent QAC mixtures against Salm. typhi

(Davis, unpublished data)

Addition	Kill (%) with detergent-QAC mixture no.		
	1	2	3
None	99·998	99·975	99·985
	99·852	99·999	99·961
	99·999	99·985	99·922
Peptone (0·5%)	99·998	99·997	90·9
	99·992	99·840	99·977
	99·999	99·970	99·961
Milk (4%)	99·967	75·2	83·2
	99·995	72·2	88·1
	99·999	70·0	86·2

The effect of organic matter depends not only on its nature and concentration but on the test organism,

TABLE V

The effect of organic matter and alkali on the bactericidal activity of four QACs under standard conditions

Organism	Additions	Kill (%) with QAC* no.			
		1	2	3	4
Staph. aureus	None	>99·997	>99·995	>99·997	>99·996
	Milk (8%)	>99·995	>99·994	>99·950	>99·995
	Na_2CO_3 (0·2%)	>99·994	>99·993	>99·996	>99·991
	Milk (8%) + Na_2CO_3 (0·2%)	>99·983	>99·967	>99·950	>99·950
E. coli	None	98·300	99·000	99·540	99·890
	Milk (8%)	88·500	87·300	83·200	82·200
	Na_2CO_3 (0·2%)	99·995	99·999	>99·999	99·99
	Milk (8%) + Na_2CO_3 (0·2%)	99·999	99·993	96·400	83·000

* The QAC concentration was always 1:13,710.

the QAC in question, temperature, time and other factors. Therefore, while we suggest that bulk whole milk is probably the best for standardization purposes, we emphasize that parallel tests with the relevant organic matter should also be made.

(vi) *Osmotic pressure*. Most disinfectant tests are made with very dilute solutions of germicide and as organic matter, even at a 2-4 per cent level, does not affect osmotic pressure significantly it is unlikely that differences in osmotic pressure encountered in practice will affect the result. However, in special techniques consideration of this factor may be required.

(vii) *Detergent-sanitizers*. Many QAC preparations are now sold as detergent-sanitizers. These generally consist of QACs compounded with non-ionic detergents or mild compatible alkalis, or both. Such preparations are tested at the dilution recommended by the manufacturers, e.g. 1:300. Two of the major factors affecting the tests, pH and concentration, are therefore automatically standardized.

During their use, e.g. for washing dishes, the disinfectant action of the solution steadily decreases for four reasons. First, there is loss of QAC through 'carry-over' and/or dilution (up to 50 per cent or more). Second, the organic matter content increases (from 0 up to 0·5 per cent dry matter or more). Third, there is a fall in pH, e.g. from 11 to 10 and fourth, the temperature drops, e.g. from 120° to 100°F. It follows that any test made on a fresh solution gives only part of the vital information. In assessing the value of any cleansing preparation (i.e. one which it is claimed will both clean and sterilize) tests should always be made on the solution both at the beginning and the end of a run (cf. Resuggan and Davis, 1947; Davis *et al.* 1949).

While the general public usually thinks of the sanitizing process chiefly in terms of the killing of the bacteria by chemicals it should be remembered that 90-99 per cent of the bacteria are removed from the equipment by the cleaning process. Chemical disinfection is only a 'finishing' process.

When testing the effectiveness of detergent-sanitizers either the wash water itself, as in dish or glass washing may be plated, or the utensils, e.g. dishes or glasses, may be swabbed. Both methods give results of the same order (Davis and Resuggan, 1946).

As might be expected, incorporation of an alkaline detergent greatly increases the effectiveness of most QACs, not only against Gram-positive bacteria but especially against *E. coli* and other Gram-negative organisms (Table V).

Considerable attention has been paid to the testing of detergent-sanitizers in the U.S.A. and a standard method, based on collaborative studies, has been reported on by Ortenzio (1958). A reduction of 99·999 per cent in 30 sec. at 25° is required (see section 4, a, iii).

While the addition of ordinary alkalis (NaOH and Na_2CO_3) may enhance the bactericidal power of most QACs, sufficient residues of known incompatible detergents (section 4, d, iv) may be present under practical conditions seriously to reduce the germicidal effect of subsequently applied sterilants. Thus soap residues may inactivate QACs, and non-ionic detergent residues may inhibit phenolic germicides. A point often overlooked is that alkali-treated fat-soiled surfaces may retain soaps (resulting from hydrolysis) and so reduce the efficiency of a QAC. This effect does not occur in the absence of fat (Ortenzio *et al.* 1954).

(c) *Inactivation*

All tests of disinfectants involve action for a defined time, and good technique requires that the time shall be measured with sufficient accuracy, e.g. ±1 per cent. With methods involving withdrawal of an aliquot after a few minutes and examination by a plate count or for surviving cells, it is obvious that the germicide is withdrawn with the suspension and continues to act, even though diluted 10 or more times. The simplest way to stop the action of the disinfectant is to add an innocuous substance which neutralizes its action or destroys it. Commonly used materials are named in Table VI. The times of reaction of these are obviously different; only a few are virtually instantaneous in action.

TABLE VI

Substances commonly used as inactivators of germicides

Germicide	*Inactivator*
Chlorine or hypochlorite	Sodium thiosulphate
Formalin	Ammonia
Phenols	Charcoal; ferric chloride
Hydrogen peroxide	Catalase
Mercurials	Sodium thioglycollate
Mercuric chloride	Sodium sulphide
QACs	Lecithin; stearate; Lubrol W
Sulphonamides	*p*-Aminobenzoic acid
Penicillin	Penicillinase
Streptomycin	Glucose
Hexachlorophene	Tween 80 (Lawrence and Erlandson, 1953)

It is well recognized that QACs may be adsorbed to bacterial cells and prevent them from growing, but not kill them during the test period. Removal of the adsorbed QAC within 10-30 min. may result in resuscitation of the cell (Valko and Dubois, 1944). However, this phenomenon is highly variable, and depends on the type of bacterium used, the QAC and the inactivator. There is no clear cut distinction between bacteriostatic and bactericidal action, or between ineffective disinfection and 'reversible dying' (cf. Marshall and Krenoff, 1937; Price, 1950; Cook, 1954). But it has become standard practice to add an efficient inactivating agent to the disinfection mixture at the end of the test period. Lecithin (Baker *et al.* 1941*b*), stearate, Lubrol W, suramin sodium (Lawrence, 1947, 1948) and aryl sulphonic acid condensate (Goetchius, 1948) have been used, and even a natural substance such as milk in high concentration. Meanwell (pers. comm.) found that 10 per cent of milk was satisfactory for inactivating QACs in tests with *E. coli*. Such compounds may revive Gram-negative but not Gram-positive bacteria (Klein and Kardon, 1947) and this procedure obviously effects an artificial distinction between cells which are already killed or irretrievably dying, and those which would die if the adsorbed QAC were not inactivated.

When QACs are used in practice we do not immediately treat the bacterial cells by this inactivation procedure. It follows that those cells which are 'reversibly dying' will die some time after the end of the treatment period, e.g. 10 min., and can therefore hardly be considered dangerous to health. It may be debated which picture—the result with, or the result without inactivation—is more closely related to practical applications of quaternary germicides (cf. Rahn, 1947).

(d) *Other factors*

(i) *Errors in measuring*. Volumetric measurement of any solution of a surface active substance is potentially subject to error. The simplest approach to the practical problem is to assume that all glassware and other objects coming into contact with the solution become coated with a mono-molecular film. All pipettes, flasks etc. should therefore be thoroughly rinsed with the solution before use. With very dilute solutions large volumes should be used in order to avoid any significant change in concentration because of this adsorption.

Obviously the greatest care should be taken in cleaning all glassware and any other equipment before use. Consecutive treatments with soap or Teepol, chromic acid, sodium hydroxide and hydrochloric acid, with very thorough rinsing between treatments and finally, should be adequate (cf. Cousins, 1951).

(ii) *Solubility*. Some otherwise efficient germicides may be limited in usefulness because of their low solubility. An interesting phenomenon that we have encountered is the increased germicidal activities of the slurries which result from the addition of germicides beyond their solubility limits. There are various possible explanations for this, but its importance lies in the fact that with the addition of say 100 p/m of a germicide whose nominal solubility is 10 p/m there may be a significant increase in the disinfectant action over that obtained with 10 p.m. Organic substances of low solubility in water are usually readily soluble in acetone and alcohol, and we have found that an alcoholic solution gives a finer slurry when dilutions are prepared in the usual way.

(iii) *Stability*. All QACs appear to be quite stable in solution, whether heated or held for several years at room temperature (Heineman, 1937; Lawrence, 1950, p. 53; Valko and Dubois, 1945; Mueller *et al.* 1946). Freezing is also without effect (Dunn, 1937; Lawrence, 1950, p. 54).

(iv) *Incompatibilities*. Many anionic compounds neutralize or precipitate QACs and are therefore incompatible in sterilizing formulations (Domagk, 1935; Hornung, 1935; Baker *et al.* 1941*a*,*b*; Valko and Dubois, 1944; Rahn, 1946). Valko and Dubois showed that apparently killed bacteria could be revived by the application of a large molecule anion within 10-30 min. However, not all anionic substances are efficient for this purpose (Lawrence, 1947, 1948). Some incompatibilities are surprising (cf. Lawrence, 1950, p. 25). Thus soluble nitrates may be incompatible with the benzyl QACs because of the low solubilities of the QAC nitrates.

(v) *Associative effects*. The narrow spectrum of some surface active germicides, particularly the QACs, naturally prompts investigations of the possibilities of mixtures of germicides. The results are rarely promising because it is difficult to find two germicides which are complementary in respect of the bacterial genera on which they act. Most disinfectants have either a wide spectrum or similar narrow spectra. It is difficult to find, for example, two germicides both having desirable properties, e.g. absence of odour, corrosive power and toxicity, one of which is particularly effective against Gram-positive bacteria and the other against Gram-negative organisms.

Any harmless substance which enhanced the germicidal power of a QAC would be of great value. Unfortunately some useful concomitants, e.g. non-ionic detergents, may weaken them. It has been claimed that some new types of compounds may enhance the bacteri-

cidal power of some QACs, and such a development would be of the greatest interest. The possibility of both synergistic and antagonistic effects should always be kept in mind when testing mixtures.

(vi) *Mixing techniques*. Apart from possible errors in timing in short tests, e.g. those of 2 min. duration, and errors in the transfer of germicide, the method of mixing can affect the result. Thus the addition of a small volume of cell suspension to a large volume of germicide may give a higher kill than the addition of a small volume of germicide to a stabilized cell suspension.

The vigour of shaking can also affect the results by breaking up cell clusters, intensifying the 'washing effect' on cells, and by producing foam. Cousins (1951) found that vigorous shaking often gave a higher percentage kill.

The apparently innocuous procedure of adding an aliquot of a broth culture to a germicide solution and making colony counts at intervals is not so harmless as it appears. We have found that kills up to 50 per cent can be obtained purely by the dilution effect, presumably due to the shock induced by the transfer of the cells to the new environment or to the washing of metabolites away from the cell surface.

(vii) *Contact time*. The periods of contact used in the recognized disinfectant tests are shown in Table VII. Working with QACs, Mallmann and Hanes (1945) used contact times of 1, 5, 10 and 30 min., Berry (1952) used 1 to 480 min. and Cousins (1951) used 2 min.

TABLE VII

A comparison of the contact periods used in standard tests

Method	Contact periods (min.)						
Rideal-Walker	2·5	5	7·5	10	—	—	—
F.D.A. (U.S.A.)	—	5	—	10	—	15	—
Hygiene Laboratory (U.S.A.)	—	5	7·5	10	12·5	15	—
Chick-Martin	—	—	—	—	—	—	30

In practice a disinfectant should achieve its objective in as short a time as possible. It seems to be generally accepted that a contact time of not less than 2 and not more than 10 min. should be used, i.e. there should be a reasonable minimum time for the germicide to act, and a maximum time acceptable in relation to such practical considerations as conveyor throughput, rinsing and staff amenities. It appears to us that these times are reasonable and suitable from all points of view. A possible criticism is that the error of timing for the addition of germicide and the withdrawal of an aliquot must be about 1 sec., and this is about 1 per cent of 2 min. However, the error of the plate count is such that an incidental error of 1 per cent is not important. Where circumstances permit contact to continue for several hours (e.g. overnight), appropriately lengthened times can be used.

The usually accepted formula relating time of reaction, concentration of germicide and number of survivors is $KC^n t = \log (a/b)$, where K is a constant; t, the time; C, the concentration; a, the initial number of organisms; b, the number after time t, and n is the concentration coefficient. However, Jordan and Jacobs (1944, 1945) showed that the logarithmic death rate (i.e. a linear relation between log survivors and time) does not really hold, and that the death rate is rather slow at first, rises to a peak and then declines to an approximately linear value. They related disinfection time to germicide concentration by the formula $C^n t = K$, and were of the opinion that measurements should be based on a high mortality. In their work they were able to determine the 99·999999 per cent mortality time, with survivor levels of about three viable cells/ml.

(viii) *Special requirements for skin disinfectants*. The need for a satisfactory disinfectant for skin has long been realized (Senn, 1905). Such a disinfectant must not only be effective against common infective agents; it must be non-irritant and penetrative. It has been claimed that phenylmercurio dinaphthylmethane disulphonate (Penotrane meets these demands) (Reid *et al.* 1958; Goldberg *et al.* 1950). It is said to be adsorbed on to the skin so that its local concentration builds up, and its activity is alleged to be enhanced in the presence of protein material such as blood, serum and pus (Reid, 1959). The latter claim is suprising in view of the fact that all other disinfectants, including mercurochrome (sodium dibromhydroxy-mercurifluorescein) are less effective in the presence of blood serum. Indeed, this is fundamentally the reason for the failure of the carbolic spray type of treatment (Lister, 1867).

In spite of the low toxicity of surface active germicides, little work has been done *in vivo*. Brewer and McLaughlin (1951) showed that benzalkonium chloride was more effective than other commonly used antiseptics for preventing the onset of tetanus in artificial infection experiments with spores of *Cl. tetani*.

(*To be continued.*)

Exhibition Lectures

With this issue we are publishing the first three of the 12 Exhibition Lectures presented during the recent LABORATORY APPARATUS AND MATERIALS EXHIBITION.

We have received many requests for copies of the journal containing specific lectures and if you are interested in obtaining any of these, we suggest that you make application now.

The remaining nine lectures are:

Recent Advances in Techniques at the Tin Research Institute.
Recent Advances in Forensic Laboratory Techniques.
The Electron Microscope in Studies in Protein Synthesis.
The Biological Assay of Insecticides.
Automation in the Medical Laboratory.
Laboratory Workshops.
The Weisz Ringoven Technique in Microchemistry.
Modern Methods for the Microbiological Examination of Foods.
The Examination of Food Additives.

ULTRA LOW-CONDUCTIVITY WATER

APPLYING an electrophoretic ion-exclusion technique, the National Bureau of Standards[1] recently succeeded in preparing water of extremely low ion content. The water obtained has an electrical conductivity of 0·039 × 10^{-6} ohm^{-1} at 18°C., indicating a residual ion content which is equivalent to a sodium chloride concentration of one part per billion. Containing less than one-third of the ionic impurities of the water prepared by Kohlrausch and Heydweiller[2] in their historic purification experiments, this water approaches the theoretical conductivity—and ideal purity—more closely than any values previously reported in the literature.

Water, because of its abundance, its importance to the physical sciences, and its role as a life-supporting liquid, has been the subject of intense study for many years. Yet, despite all this research, it apparently had not been produced previously with such a small ionic content.

The present research, undertaken by Wolfgang Haller and H. C. Duecker of the Bureau's staff, was part of a study on ion diffusion. In designing the purification procedure, two principal considerations were involved. First, instead of using multiple distillation as in the classic experiments, an electrophoretic procedure was applied. In this way purest water was obtained in two hours, while in the best previous study,[2] 36 consecutive vacuum distillations were required. Second, the purification apparatus was designed to recirculate the purified water continuously through the electric field, thus immediately removing any ions which might originate from the walls of the apparatus and contaminate the already purified water.

By this kinetic method, it is possible to maintain the purity of the water if a container material with very low ion-release is used. While fused silica containers which had been exposed to water for ten years were utilized in former purifications,[2] high-density polyethylene, platinum, and borosilicate glass which had been exposed to steam for a number of hours just before purification were found suitable for the present experiments. The steam condenses in the apparatus, thus rinsing its internal surfaces with a stream of freshly distilled water. At the temperature of this treatment, the diffusion of ions from the glass—which is the main potential source of ionic impurities—is many times faster than at the temperatures at which the apparatus is used, resulting in an effective depletion of the surface regions of the glass from objectionable ions.

Purification

In the purification process, an electric field causes anionic and cationic impurities to migrate from water in the centre part of a purification cell through two ion-selective membranes, thus depleting the water in the centre compartment of ionic impurities. The water is continuously recirculated by a platinum impeller pump through the electric field of 1,000 v./cm., which is maintained between the surfaces of the two membranes. Other components in the pure water loop are a heat exchanger, a conductivity cell, and a thermometer well. The surfaces of the ion-selective membrane which are opposite to the pure water, are in contact with electrolyte solutions (0·05 N ammonium hydroxide and 0·02 N sulphuric acid), which are connected by platinum electrodes to a stabilized d.c. source. Both electrolytes are circulated through separate loops by a multichannel peristaltic pump. These loops contain heat exchangers and storage reservoirs.

The rapid flow of the electrolytes provides a homogeneous ion distribution in the region between the membranes and the platinum electrodes. If this circulation is not maintained, the voltage gradient in this area decreases the electrolyte concentration next to the membranes. Then the electrical resistance between membranes and electrodes increases, leading to a drop in effective membrane-to-membrane voltage which is essential for the purification process. The rapid flow past the electrodes also reduces any gas polarization of the electrodes because the gas bubbles are carried into the reservoir where they can escape.

Data on Ultra Low-Conductivity Water Obtained by Various Workers

Worker	*Conductivity in* 10^{-6}ohm^{-1}cm.$^{-1}$	*Temperature*	*Conc. (in p.p.m.) of a sodium chloride solution with same conductivity*	*Method of purification*
		°C.		
Haller and Duecker[1]	0·0390	18	0.0010	Electrophoresis
	0·0589	25	0·0018	
Kohlrausch & Heydweiller[2] ...	0·0429	18	0·0031	Distillation
	0·062	25	0·0032	
Bengough, Stuart, & Lee (1927)	0·045	18	0·0043	Do.
Kraus & Dexter (1922) ...	0·048	18	0·0059	Do.
Weiland (1918)	0·053 to 0·066	18	0·0086 to 0·0156	Do.
Bencowitz & Hotchkiss (1925)	0·06 to 0·07	Not given	—	Do.
Thiessen & Herrmann (1937) ...	0·065 to 0·080	25	0·0046 to 0·0118	Do.
Kunin & McGarvey (1951) ...	0·07	Not given	—	Ion exchange
Gostkowski (1934)	0·07	0	0·0280	Distillation
Hulett (1896)	0·081	Not given	—	Do.
Bourdillon (1913)	0·086	18	0·0260	Do.
	0·0371	18	0·0000	
Pure water (theoretical) ...	0·0549	25	0·0000	
	0·0111	0	0·0000	

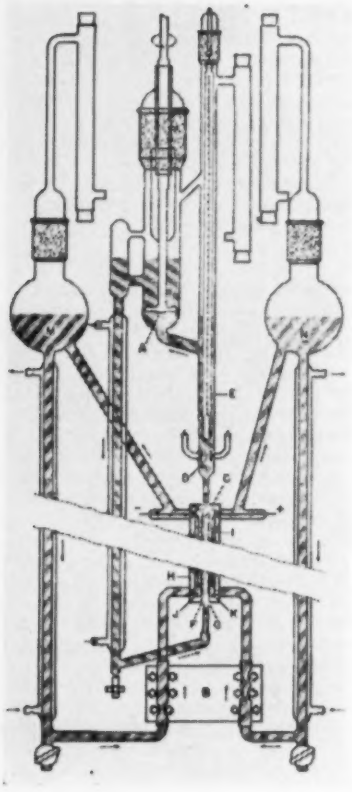


Fig. 1. Schematic diagram of apparatus developed by the National Bureau of Standards for preparing water with extremely low ion-content. By using this modified electrophoretic equipment, ultra low-conductivity water with a residual ionic impurity content of only one part per billion is obtained. The apparatus includes an (A) impeller pump, (B) peristaltic pump, (C) centre compartment, (D) conductivity cell, (E) thermometer well, (F) cation permeable membrane, (G) anion permeable membrane, (H) cathode, (I) anode, (J) cathode compartment, (K) anode compartment, (M) cathodal reservoir, and (N) anodal reservoir.

The conductivity measurements are made in a carefully calibrated conductivity cell, located in the pure water loop at the exit of the purification cell. A limiting purity is reached after about two hours of operation.

Dissociation Constant

With the postulation of the theory of ionic dissociation in the late 1800's, early workers found it convenient to measure the electrical conductivity of ion-free water to obtain quantitative data of the dissociation equilibrium of water. However, because research carried out in this period led to values which later had to be corrected, today's best values of the dissociation constant are based on indirect methods, which do not necessitate the preparation of ultra-pure water. As the Bureau's low-conductivity water more closely approaches ultimate purity than any water reported before, and as more correct supporting electrochemical data are available today, Bureau scientists have been able to apply the electrical conductance approach to measurement of the dissociation constant of the water[3].

REFERENCES

[1]For further technical information, see Ultra low-conductivity water by electrophoretic ion exclusion, by Wolfgang Haller and H. C. Duecker, J. Research NBS, 64A (Phys. and Chem.), 527 (November-December, 1960).

[2]F. Kohlrausch and A. Heydweiller, Wied. Ann. 53, 209 (1894); Z. physik. Chem. 14, 317 (1894).

[3]Determination of the dissociation equilibria of water by a conductance method, by H. C. Duecker and W. Haller, in process.

FIRE PROTECTION IN THE LABORATORY

THE difficulties associated with protecting laboratory and research areas against fire outbreak, particularly when overnight tests are left running without supervision, has caused Imperial Chemical Industries Limited, Plastics Division, to install Auto-thermatic fire detection at their Welwyn Garden City headquarters.

The actual buildings covered include laboratories for research and development plus a general stores area, all of which are grouped on one part of the site. Each department officially closes at 5 p.m. from which time they are supervised only by site security men whose calls of inspection are nevertheless insufficiently frequent to guarantee that any outbreak of fire would be detected.

Although there are no particularly hazardous elements classified in the areas protected, apparatus may be left running through the night and, in the event of mishap, these tests could in turn be responsible for sparking off a fire.

A major feature which had to be borne in mind when a detection system was selected was the flexibility of the detector layout. The laboratories at the Welwyn site have been built to be adjustable in layout. The buildings are designed on a 4 ft. module and all partitions can be adjusted at will on this module. Even lighting arrangements are of the plug-in type. To have a fixed fire detection system would to a large extent minimize this flexibility since a change in building layout could render the detection system less effective. Since the Auto-thermatic system is designed for use out of conduit, however, it is a simple matter for the company electricians to make any changes necessary in the positioning of detectors.

The system used is the Mark IV Auto-thermatic type which has over 30 detector sections, 22 of which have been connected for immediate use. Future extensions can be made at a later date without any expensive additions of control equipment. When these additions are necessary, also, the site electricians will be able to handle the job themselves, thereby cutting down on labour costs.

The types of detector used include the Dimac fixed head detector and break link cable type. The break link cable is used to span the larger roof areas of the stores building, which is a reinforced concrete structure of single storey design.

The stores area is used for general purposes and accommodates machinery, materials and general supplies. Adjacent, however, is a paints store which is in turn protected by a CO_2 installation which is connected to the Auto-thermatic system. An immediate indication is given at the central control when the CO_2 system is triggered off.

The analytical laboratory at the I.C.I. Plastics Division, Welwyn Garden City, together with many other departments is protected against possible fire outbreak by Auto-Thermatic fire detection.

Bench research is the primary interest of the research laboratories which are three storey constructions having steel frames and concrete floors, with curtain walling. Property contained in this building is almost exclusively scientific.

The approximate floor areas now protected by Auto-thermatic are about 180,000 sq. ft. altogether.

The entire section of the site occupied by these buildings is served by a ring main run from the central control equipment. From this main run 22 detector sections. Further detector sections can be connected to the ring main as and where required.

Each detector section of the Auto-thermatic system operates through a pulse box situated within the building covered. It is also possible to site them on outside walls, depending upon the planned method of usage. An additional function of the pulse boxes is to provide a plugging-in point for a normal telephone handset so that conversation can be conducted throughout the entire area of fire cover. A fireman attending a detected outbreak can quite simply report back to central control by plugging in the handset, irrespective of its location. This is particularly useful in those areas where telephones are not normally sited.

The Works Fire Brigade forms a nucleus force to look after any outbreak as a first line of defence, both day and night. The detection system now installed should give a warning immediately a fire breaks out. This will save valuable time and make sure that a local attacking force can be quickly mobilized and directed to the fire.

Hand extinguishers are placed throughout the entire site and there are several $2\frac{1}{2}$ in. external fire hydrants for use by the Hertfordshire Fire Brigade.

The Hertfordshire Fire Brigade receives a call automatically via a direct line to its Welwyn Garden City Fire Station whenever a fire detector is triggered off. The gateman at the entrance to the site receives a signal at the same time so that he can make sure there will be no obstacles in the way when the Brigade arrives.

Situated only one mile away, the Brigade can be on site within a very short time of actual outbreak, particularly now that automatic detection has been installed.

The Auto-thermatic system is manufactured by Sound Diffusion (Auto-thermatic) Ltd. Similar installations are now in use at the I.C.I. offices at Billingham and at the associated company British Visqueen Ltd., Stevenage.

Redetermination of the National Carbon-14 Standard

THE value of the national sodium carbonate-C^{14} standard has been redetermined with increased accuracy at the National Bureau of Standards.[1] The revised value of the standard is 1250 ± 1.5 per cent disintegrations per second per gram of solution, as compared with a value of 1280 ± 10 per cent dps/ml. of solution obtained in 1949. The redetermination was made by W. B. Mann, H. H. Seliger, W. F. Marlow, and R. W. Medlock of the Bureau's radioactivity laboratory.

Developments in low-level counting techniques are making possible their application to research in chemical, biochemical, and industrial processes having rigid requirements that cannot be met by ordinary laboratory procedure. Recently developed equipment will be capable of more precise measurement only to the extent of the accuracy of its calibration. The redetermined value of carbon-14 radioactivity is useful in these areas as well as in such extensive fields as metabolism studies based on tracer techniques and geological and archeological dating.

The Bureau in 1949 issued the first national carbon-14 standards in the form of sodium carbonate-C^{14}. These standards were measured by counting the carbon in the form of carbon dioxide in length-compensated internal gas counters in the Geiger region. The stated uncertainty in these standards was ± 10 per cent, reflecting possible wall effects (of the order of 2 per cent) and a lack of agreement (about 15 per cent) among results from the National Bureau of Standards and two other laboratories participating in comparative measurements. Discrepancies as large as 15 per cent were subsequently reported between results obtained in the proportional and the Geiger regions.

The objects of the present investigation of the carbon-14-standard disintegration rate were to examine and resolve these discrepancies, and to determine the conditions under which carbon-14 labelled carbon dioxide could be counted with nearly 100 per cent efficiency in the same counters in both the proportional and the Geiger regions. These objectives were accomplished within the limits of experimental error, and the uncertainty in the value of the NBS sodium carbonate-C^{14} standards was reduced by an order of magnitude.

The redetermined sodium carbonate-C^{14} standard sample may be obtained from the Radioactivity Section, National Bureau of Standards, Washington 25, D.C. for $27.00.[2]

REFERENCES

[1]For further information, *see* 'Recalibration of the NBS Carbon-14 Standard by Geiger-Müller and Proportional Gas Counting,' by W. B. Mann, H. H. Seliger, W. F. Marlow, and R. W. Medlock, Rev. of Sci. Inst., July, 1960.

[2]A complete listing of standard samples is contained in Standard Materials, NBS Circular 552 (third edition), available from the Superintendent of Documents, U.S. Government Printing Office, Washington 25, D.C. (35 cents).

Water Pollution Research Laboratory

The Water Pollution Research Laboratory will be holding Open Days on Wednesday and Thursday, October 11 and 12, 1961. The laboratory will show its work on development of methods of treatment of sewage and industrial wastes, and on other aspects of pollution.

Applications for invitations to visit the laboratory on these days should be sent to the Director, Water Pollution Research Laboratory, Elder Way, Stevenage, Hertfordshire.

News from the British Association of Chemists

DEATH OF MR. NORMAN SHELDON

By the death on July 18, 1961 of Mr. Norman Sheldon, A.R.C.S., F.R.I.C., the B.A.C. has suffered a most grievous loss.

Born in Walsall and educated at Heversham, Westmorland, Mr. Norman Sheldon took his A.R.C.S. in Chemistry at the Royal College of Science in 1914, during which time his scientific training was complemented by business training in his father's factory.

After experience in the acid industry and as research chemist in the steel industry, Mr. Sheldon, in 1923, became interested in the production of scientific glassware and spent some time with Duroglass Ltd., of London. Research work in this direction led him to originate the manufacture of 'Monax' glass by John Moncrieff Ltd., of Perth, and in 1925 he became associated with the laboratory porcelain department of the Worcester Royal Porcelain Co., Ltd. He had completed 36 years as Sales Organizer for these organizations.

In the period between the wars, realizing the danger of German competition in the glassware and laboratory porcelain industries, Mr. Sheldon began a campaign on behalf of the industries in this country, designated as 'Key Industries', which played a great part in ensuring the continuation of production in Great Britain. He has lost no opportunity in stressing whenever possible that Britain must lead in the production of every kind of scientific equipment if the future of her industries is to be assured. Mr. Sheldon's articles and letters in the technical and daily Press on this and other topics have been numerous.

In 1940 he was elected chairman of the British Chemical Ware Manufacturers Association, still held at the time of his death, and he was also a Member of the Committee of The British Lampblown and Scientific Glassware Manufacturers Association.

Mr. Sheldon's association with the B.A.C. began in 1917, when he became a founder member. In the early years of his membership his activities were confined to the recruitment of new members. Following his election as member of the London Section in 1943 Mr. Sheldon immediately took a great interest in the organization of Section meetings, and he was responsible for the first big open meeting held by the London Section. In the same year he opened a discussion on 'Safeguarding the Master Key Industries' at which Lord Strabolgi, the late Dr. E. F. Armstrong, and Col. W. A. Bristow were present. The report of this meeting was published, and copies sent to every member of the Government of the day. This publication contributed to the awakening of the authorities to the importance of the small industries making laboratory equipment.

In 1946 Mr. Sheldon organized, for the London Section of the B.A.C., a lecture by Mr. R. E. Threlfall, M.A., F.S.G.T., on glass tubing which was attended by many distinguished members of the chemical profession and leaders of the scientific glassware industry. This was so interesting that there was an immediate request for the lecture to be available in a permanent form. The publication 'Glass Tubing' was issued by the B.A.C. therefore and there was a very considerable demand for copies. Requests for it are still being received today.

Mr. Sheldon served the B.A.C. continuously for over 15 years as President (three years), Vice-President, or Member of Council, as well as being a member of the Unemployment Benefit Fund, Publications, Legal and Parliamentary Committees and the B.A.C. representative on the Parliamentary and Scientific Committee. He was also the leading spirit in the acquisition and fitting up of Hinchley House, Harley Street, London, W.1. as the Headquarters of the Association and was Chairman of the Committee responsible for its supervision and upkeep. This recital of offices held however, represents only a summary of the vast amount of work which he did, not only for the Association, but for individual members and the staff. In 1951 this was recognized by the award to him of the Association's Hinchley Medal.

In addition to all his professional activities Mr. Sheldon devoted a large part of his energies to Local Government. He had been a Member of the Twickenham Council since 1946 and a Member of the Middlesex County Council since 1958.

As a person Norman Sheldon was a man of the utmost sincerity and integrity. In support of any cause which he thought just he was an uncompromising fighter, of intelligent persistence and refusing utterly to accept defeat. At the same time he was a most modest, kindly and courteous person, filled with a compassion for people. He spent much of his time and money on others and a large part of his work will remain unknown except to those whom he helped. His death is a great loss to the community and, in the words of a leading article in the *Richmond and Twickenham Times* ' . . . the sparks of life he struck are unquenchable and the good he did will not be scattered with his ashes.'

Annual General Meeting

At the 43rd Annual General Meeting of the Association held at the Euston Hotel, London on April 22, 1961, the following officers were elected for 1961/62:

President	H. L. Howard, B.Sc., A.R.C.S., D.I.C., F.R.I.C., M.I.Chem.E.
Vice Presidents	J. Wilson, C.B.E., M.C., M.Sc., F.R.I.C., F.I.R.I.
	R. J. Mollard
	L. E. Puddefoot, B.Sc.
Hon. Treasurer	A. T. Healey, Ph.D., D.I.C., F.R.I.C., A.M.I. Chem.E.
Hon Secretary	E. H. G. Sargent, A.C.G.F.C.
Hon. Registrar	J. E. Gregory, B.Sc., Ph.D., A.R.I.C.
Trustees	J. Greenwood, J.P., A.M.C.T., F.R.I.C.
	T. McLachlan, A.C.G.F.C., F.R.I.C., M.I.Biol.
	H. T. F. Rhodes, M.I.E.I.
Hon. Editor	H. Warson, B.Sc., F.R.I.C.
General Councillors	C. H. Bowyer, B.Sc., F.R.I.C., A.C.I.P.A.
	G. Robertson
	H. Warson, B.Sc., F.R.I.C.
	C. J. H. Wylie.

Section Councillors

Birmingham:	Mrs. S. M. Tritton, M.P.S., F.R.I.C.
	H. T. Candy
Manchester:	H. Batley, B.Sc., A.I.M.
	J. Rickman
Yorkshire:	G. C. Woodward
Liverpool:	T. Q. Matthews
Notts. & Derby:	A. J. Wesson
	J. Neilson, B.Sc.
London (Area 1)	E. G. P. Burwell
	R. E. Love
London (Area 2)	G. H. Clarke
	W. A. Phillips

All correspondence and enquiries concerning this Association should be addressed to G. R. Langdale, Esq., The British Association of Chemists, Nomination Dept., 14 Harley Street, London, W.1. Tel.: Museum 7021.

LABORATORY EQUIPMENT & APPLIANCES

New 'Top-Pan' Precision Balance

A new precision balance with several outstanding features, including a special out-of-level device which entirely, obviates the need for critical levelling, has been introduced.

The new balance—the Sauter Top-Pan—is a single-pan balance designed on the substitution weighing principle providing constant sensitivity. The large, interchangeable load pan, available in chromium-plated brass or in stainless steel, is mounted on the top of the instrument, where it is freely accessible. Clear, illuminated scales are featured back and front of the instrument, facilitating checking by a second operator. The balance incorporates near-instantaneous magnetic damping for really rapid operation.

The Top-Pan is available with capacities of 1 kg. (graduated with 0·1 g. scale divisions), 2 kg. and 4 kg. (both with 1 g. scale divisions). A special model is also available without the out-of-level device but with a built-in taring facility, a signal lamp showing when this facility is in use.
(Manufacturers: Shandon Scientific Co., Ltd., 6 Cromwell Place, London, S.W.7).

Cobalt 60 Irradiation Units

Two new irradiation units, of relatively low cost, for laboratory experiments with Cobalt 60 radiation—the Gammacell 100 and 200—have been announced by Atomic Energy of Canada Ltd.

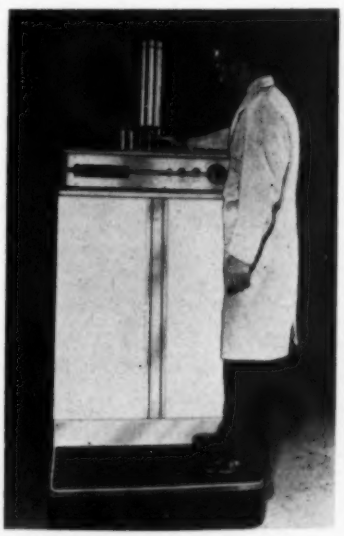
The Gammacells 100 and 200, which are similar to the model 220 announced last year, have a fixed ring of encapsulated Cobalt 60 rods into which the specimen is moved. The ring of Cobalt 60 rods is completely surrounded by a lead shield—no additional protection or special room is required.

The cell volume of the Gammacells 100 and 200 are 1½ in. diameter × 4½ in. high, and 3½ in. diameter × 5½ in. high respectively. Dose rates of up to 3×10^5 r./hr. in the centre of the irradiation chamber are available.

Operation is by electric motor and timer. The specimen is placed in the sample chamber which is lowered into the ring of Cobalt 60 rods; at a pre-set time the sample is withdrawn. Access tubes are provided—one on the model 100 and two on the model 200—to take electrical connections, coolant, etc. Additional experiments can be carried out in a helical tube surrounding the source.

In no way is it possible to receive an overdose of radiation. Dose rates outside the cell are very low—less than 5 mr/hr., 12 in. from the surface using the largest source.

The Gammacell is used in research establishments in many parts of the world. The new models 100 and 200 will allow a programme of irradiation experiments to be carried out on a quite modest research budget.
(Available from: Watson & Sons (Electro-Medical) Ltd., Industrial Division, East Lane, North Wembley, Middx.)



Auto-scanner for Bi-dimensional Paper Radiochromatograms

This is an electronic instrument for the quantitative evaluation of bi-dimensional radiochromatograms using beta-emitters such as C^{14}, P^{32}, S^{35}, and with accuracy better than 1 per cent.

The radio-chromatogram is taped to the print out paper inserted into the electric print-out machine which carries out the actual scanning by moving the chromatogram between the two end window G.M. tubes. All counts are reduced to a four figure print-out. The scanning period is variable at will up to 999 seconds.

If the counts are below the preset threshold (up to 99) the programme unit allows the scanning to be carried out in much less time than the actual preset scanning time, and the print-out is in black; unit areas with counts above threshold are allowed to be scanned for the full preset time period and the print-out is in red.

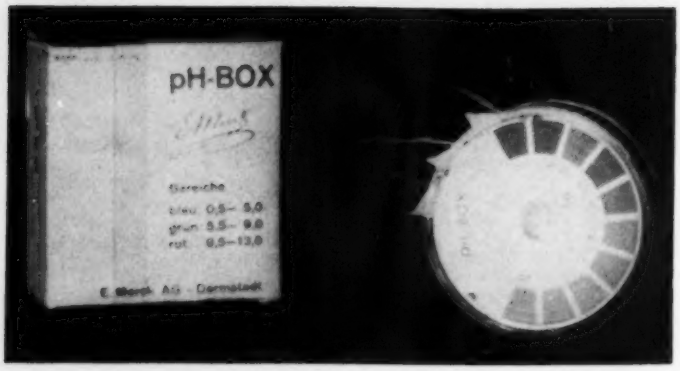
The counts are passed to the scaler from the programme unit and after the scanning period, read-out and print are initiated.

Automatic self checking is introduced as the last count on each line, from a standard frequency source.

The actual number of lines scanned is preset on a line counter which automatically switches off the whole instrument after this number of lines has been scanned.

The instrument is based on original ideas of Prof. E. B. Chain and an original design of M. Frank in conjunction with Beechams Research Laboratories Limited.
(Manufacturers: Gauging Systems Ltd., Royston Road, Baldock, Herts.)

Left: The Gammacell 200 for laboratory experiments with Cobalt 60 radiation.



The Merck pH box.

Merck pH Box

An addition has been made to the Merck range of indicator papers, i.e. the Merck pH box which incorporates three strips of indicator paper with different pH ranges. They are contained in a plastic dispenser and in different colours. The torn off strip of indicator paper is inserted for a few seconds into the solution to be tested. With highly viscous or stained liquids, and with suspensions, the substance is dripped on to the indicator paper. The wet paper (with liquids dripped on to the paper take the wet reverse side of the indicator paper) is then compared with the colour scale.

The price of this box is 17s. 10d.
(Available from: Anderman & Co., Ltd., Battlebridge House, 87-95 Tooley Street, London, S.E.1.)

Cutting Box

A prototype of the Anderman compact cutting box was on show at the recent Laboratory Apparatus and Materials Exhibition. This is a remarkably small cutting device incorporating a motor driven diamond loaded slitting wheel for cutting glass, ceramics, tungsten, carbide, etc. It will find a wide range of applications in the laboratory and is of particular use to glassblowers.

There is a sliding stop bar and a warning 'on' light. The wheel is protected by a shield and is water cooled by the provision of a drip tank. The unit measures approximately 4½ in. × 4½ in. × 14 in.
(Manufacturers: Anderman & Co. Ltd., Battlebridge House, 87-95 Tooley Street, London, S.E.1.)

Dye-Dilution Recorder

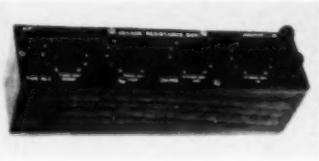
A completely redesigned Cambridge dye-dilution recorder, Mark II, for detecting and diagnosing structural and functional disorders of the heart, is now available. Full clinical tests have proved the exceptional sensitivity and stability of the instrument and the many refinements incorporated are a result of co-operation with a large number of hospitals.

In making a dye-dilution curve a small amount of non-toxic dye is injected into a vein of the patient and its concentration recorded after the mixture of dye and blood has passed through the heart. The concentration of dye in the blood is detected photoelectrically, either by a small earpiece, which clips on to the lobe of the ear, or by a cuvette through which a blood sample is continuously drawn. In each of these units a small lamp shines through the blood flow to activate a pair of photocells, and when the dye-concentration in the blood changes a corresponding variation in the cell-output is caused. This variation is recorded on a Cambridge electronic strip chart recorder, which has a response time of better than two seconds full-scale and a sensitivity and accuracy of better than 0·1 per cent and 0·5 per cent full-scale respectively. In this application minimum and maximum ranges are 0·8 mV. and 8 mV. full-scale with low and high load input impedences of 3·38 kΩ and 500 kΩ respectively.

A notable feature is the pulse rejection control, which, without over-damping or otherwise affecting the performance, positively eliminates arterial pulsations from the record. All the controls necessary for operating and testing the apparatus are situated on a panel at the front of the recording unit. They include: dye selector and lamp strength switches; servo and chart-motor switches; two coaxial knobs giving coarse and fine adjustment of the position of the pen on the chart; and an event marker which makes a small 'pip' in the record and is operated by two push buttons, one on the panel and the other at the end of a six foot lead.

For quantitative estimations two methods for linear interpretation of curves are provided.
(Manufacturers: Cambridge Instrument Co., Ltd., 13 Grosvenor Place, London, S.W.1.)

The Anderman cutting box.



Decade Resistance Box

The decade resistor box type RD2 is a switched unit covering the range 1 ohm to 10,000 ohms with four dials.

It has been designed for use primarily in development laboratories and technical colleges where medium accuracy is required, and where low price, robustness and convenience are important considerations.

Of very modest cost, this unit will also make a valuable addition to the electronic engineer's development tools whereby the resistance required in a particular circuit can be determined in a matter of seconds.
(Manufacturers: Lionmount & Co., Ltd., 40 Hatton Garden, London, E.C.1.)

LABORATORY EQUIPMENT

We are always pleased to publish news of developments in the scientific field and manufacturers are requested to let us have full details whenever available.

Mixed Bed Deionizer with Pre-purifier

The new Elgastat E.101 provides ultra pure water in pharmacy, chemistry and electronic manufacture and for general laboratory work and research, it provides purified water instantly and at high flow rates.

To achieve highest economy, the raw water is first passed through a pre-purifier (which requires occasional recharging) and this removes the bulk of the dissolved solids from the raw water. The influent then passes through a mixed bed Elgalite cartridge, which does not require regeneration in situ.

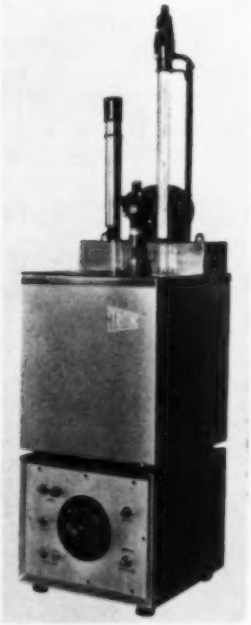
The Elgastat E.101 is fully mobile. The raw water influent point is connected to mains, and by setting the selector to Elgalized water, flow commences instantly at a flow rate of 50 gallons per hour. Regardless of raw water quality the Elgalite mixed bed cartridge will yield some 1,000 gallons of ultra pure water between cartridge exchanges.

Frequency of recharging depends upon the quality of the influent water. In London, charging takes place after some 200 gallons have been drawn, whereas Leicester will yield 400 gallons and Sheffield 800 gallons. For this purpose, standard packs of recharging fluid are supplied; the tanks are filled and by simply opening two control valves, charging takes place without supervision or attendance.

A conductivity meter indicates effluent quality throughout usage. A signal indicates when the pre-purifier needs recharging. The quality of the effluent is consistent throughout usage, purity being of the order of 10 megohm-cm. (0·01 micromho).

The assessment of effluent cost consists simply of exchange cartridge price plus charging fluid used. There are no imponderables and there is positively no labour cost. Regardless of area, cost per gallon of deionized water does not exceed 1d. and in many areas it is well below this figure. The Elgastat E.101 costs £210.

(Manufacturers: Elga Products Ltd., Lane End, Bucks.)



Thermometer Standardization Oil Bath

Designed for the ageing and calibration of large specialized thermometers for works and laboratory, this new oil bath is made of stainless steel and is of welded construction. A constant speed agitation unit is built on to the body of the bath. The heating is variable and the elements have 'no heat' sections where they emerge from the bath, thus eliminating fire risk. Silicone oils are recommended because they are non-flammable and do not vary greatly in fluidity with temperature.

For ageing operations the thermometer is immersed in the oil bath to the required depth and the bath is switched to autocycle control. The oil then heats up to a peak temperature—maximum 300°C.—at which the heater is automatically switched off, allowing the oil to cool by normal heat loss to 50°C. at which temperature the heater cuts in and reheats the bath as before.

This cycle is repeated as often as required and provided that the correct maximum temperature is pre-set, the unit may be left for an indefinite time without danger of the thermometer bursting through over-heating or accident.

After the ageing of a thermometer has been accomplished it may be calibrated and standardized in the oil bath. Normal thermostatic controls are used to hold the oil at various temperatures in the range of the thermometer being calibrated. An N.P.L. thermometer is used for the master readings. Thus a thermometer can be calibrated over its whole range and requisite corrections applied for points in the range.

(Manufacturers: E. W. Procter Ltd., Triton Works, Leeds Road, Huddersfield; Selling Agents: Ralph Cuthbert Ltd., 10-12 Westgate, Huddersfield.

The 'Varicoil' Magnetometer

A new instrument has been produced for use in the advanced phase of the new Physics for Grammar Schools Syllabus of the S.M.A. and A.W.S.T. Pilot Scheme, and for the advanced level in Physics in the G.C.E.

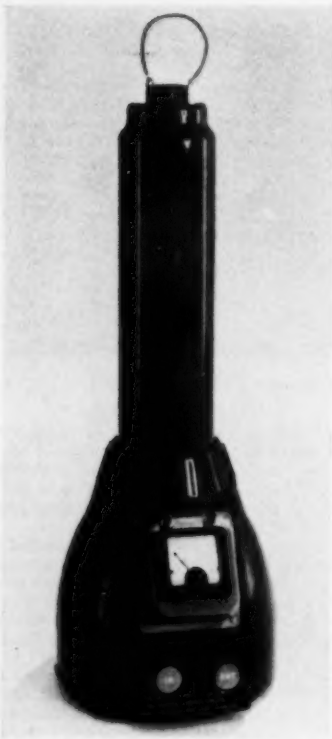
This instrument, the Griffin Varicoil magnetometer, an improved version of tangent galvanometer, enables the relationship between field and all three variables in the reduction factor to be established, where the conventional galvanometer allows only the F:i relationship.

The Varicoil magnetometer can also be used to demonstrate that, for a given current, the field at the centre of a circular field is inversely proportional to its radius; or that, for a given current, the field at the centre of a circular coil is directly proportional to the number of turns.

The magnetometer is priced at × 12 in. board carrying five concentric circular turns of different radii in the form of a printed circuit with a deflection magnetometer placed at their centre. A tapped, wire-wound circular coil concentric with the others and in the same plane has one side exposed for easy measurement of its mean diameter. It has a total of 15 turns and is tapped at 1, 3 and 7 turns so that by a selection of the terminals it is possible to obtain a wide range of turn values. The 7 in. diameter plastics base carries a series of terminal sockets labelled to correspond with the single turns and the tapped coil respectively. The leads consist of twin flex (to minimize magnetic disturbance) terminating in wander plugs which give simple and quick alterations to connections and eliminate the necessity for a reversing switch. The magnetometer rests on a slightly tapered tubular support to allow a degree of levelling to be achieved simply, easily and efficiently.

The magnetometer is priced at £15. 12s. 6d.

(Manufacturers: Griffin & George Ltd., Ealing Road, Alperton, Wembley, Middlesex.)



Portable Deminrolit Unit

The largest unit in the Deminrolit range is the Mark 7 portable Deminrolit unit. This consists of a plastic body accommodating a battery-operated tester and control valves, plus a detachable plastic cartridge containing a bed of intimately mixed 'Zeo-Karb' cation and 'De-Acidite' anion exchange materials. When water passes down through this column, virtually all ionized solids are removed, resulting in demineralized water of a quality satisfying the B.P. specification for purified water.

After treating its rated capacity, the cartridge is replaced by a regenerated one and further treatment can then continue. Permutit have a speedy cartridge exchange system to ensure continuity of supply, and the low cost of this equipment will appeal especially to the user of small quantities of pure water. The complete unit plus a spare cartridge costs £35, and replacement cartridges cost £5. 6s. 0d. for five. When it is realized that on water of London type, the unit will produce about 16 gallons of purified water per cartridge—and much more on moorland type water common in Northern England—the low cost of operation is at once apparent. Added to this there is the undoubted advantage of the ion exchange process: no scale or sludge, simplicity in operation, constant flow rates and purified water of consistent quality—monitored continuously by the tester.

The introduction of this new unit in no way affects the availability of the larger portable Deminrolit units which operate on similar principles but are equipped for regeneration on the spot. (Manufacturers: The Permutit Co., Ltd., Permutit House, Gunnersbury Avenue, London, W.4.)

The Securabrand Pipette Filler

Giving absolute safety in pipette filling, the Securabrand pipette filler incorporates seven important factors:

1. Avoids the undesirable practice of sucking liquids into pipettes by mouth.
2. Eliminates the danger of infection and poisoning.
3. The elastic mouthpiece of rubber fits easily to all normal pipettes and particularly to Glasbrand pipettes by Brand.
4. Easy manipulation with one hand by 'pulling up' on the macro type and screwing on.
5. Precision dosing and easy reading of the scale during filling and emptying.
6. Interchangeability of all parts.
7. A precision ground instrument at low cost.

Securabrand macro and micro pipette fillers are available. (Manufacturers: Anderman & Co., Ltd., Battlebridge House, 87-95 Tooley Street, London, S.E.1.)

CATALOGUES, BROCHURES & LEAFLETS

Received from Manufacturers

Pyrex Volumetric and Graduated Ware.—The manufacture, testing and some applications of volumetric and graduated glassware are described in 'Pyrex Glass', the bi-monthly technical bulletin of James A. Jobling and Co., Ltd., Sunderland. The company manufactures nearly 180 different standard items under this classification, ranging from babies' feeding bottles to graduated laboratory ware accurate to within ± 0.68 ml. in 100 ml. and carrying a National Physical Laboratory certificate. The glass has a high degree of resistance to mechanical and thermal shock, to acids (except hydrofluoric and glacial phosphoric), and to alkalis. The markings on graduated and volumetric ware are of a guaranteed accuracy.

Refractory Catalogue.—Anderman & Co. Ltd., of Battlebridge House, 87-95 Tooley Street, London, S.E.1., have issued the second edition of their Refractory Catalogue. This contains details of the refractory tubing which the company can supply together with prices for the most popular materials and sizes.

I.D.L. Leaflets.—The following leaflets have recently been published by Isotope Developments Ltd., Bath Road, Beenham, Reading, Berks.: Tritium scintillation counter 6012, pulse height analyser type 1870, timer 1860, Ratemeter 1750 and 1815, scaler 1805. Copies of these are available on application to the company and give details and specifications of the various items of equipment listed above.

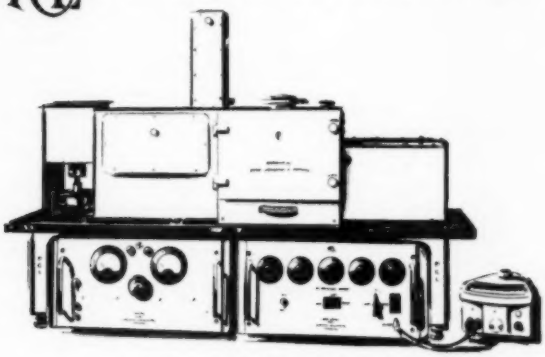
Marconi Instrumentation.—The June issue of this publication, issued by Marconi Instruments Ltd., St. Albans, Herts., contains articles on the S-band spectrum analyser, the X-band signal generator, the gamma radiation monitor, alignment of modern communication receivers using TF 144H and TF 1104, the oscilloscope pre-amplifier, the standardization of crystal calibrators and the measurement of fast pulse rise times.

B.D.H. Leaflets.—New entries in the catalogue of British Drug Houses Ltd., of Poole, Dorset, are listed in the July 1961 issue of their monthly leaflet. These are as follows: *Organic and Inorganic Chemicals*—*n*-heptane; *n*-hexane; *n*-pentane; terephthal-dialdehyde; 1:1:2-trichloro-1:2:2-trifluoro-ethane; triethanolamine.

Grey-Blue Bungs.—A new range of Grey-Blue bungs has been introduced by Jencons (Scientific) Ltd., of Mark Road, Hemel Hempstead, Herts. Full details and prices are given in a newly published leaflet which is available on application to the manufacturers.

Thermocouples.—The Cambridge Instrument Co., Ltd., of 13 Grosvenor Place, London, S.W.1. have issued a new publication (List 325) describing their standard range of rare- and base-metal thermocouples for temperatures up to 1,500°C. In general, thermocouples are manufactured to customers' specifications and for particular applications, but certain types of thermocouple assemblies, which have been continuously in demand, are now produced as a standard range. They are immediately available from stock at a lower price than comparable non-standard assemblies. The publication gives details of the factors governing the choice of suitable assemblies for widely differing applications, and describes a simple three-letter coding system which simplifies the ordering of stock assemblies and replacement parts.

PCL *A Complete Service to Polymer Science*

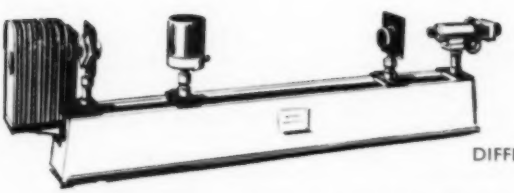


LIGHT SCATTERING APPARATUS

Polymer Consultants Limited also manufacture Light scattering apparatus, osmometers, ebulliometers, differential refractometers and a large range of pure chemicals for research for clients throughout the world.

P.C.L.—LEE
H.F. TITRIMETER

The prototype instrument used and exhibited by Dr. Lee was constructed by Polymer Consultants Limited.



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NEWS

General

The Chemistry and Microscopy of Food, Drugs and Water

The Chelsea College of Science and Technology, Manresa Road, London, S.W.3, (Department of Chemistry) announces a course on the chemistry and microscopy of food, drugs and water. Lecturer in charge will be R. G. Minor, F.P.S., F.R.I.C. and the Lecturer in Materia Medica, W. C. Bowman, B.Pharm., Ph.D., M.P.S., M.I.Biol. Assistant lecturers and demonstrators are: E. C. Clarke, F.I.M.L.T., E. J. Dyett, B.Sc., A.R.I.C., D. Pearson, M.Sc., F.R.I.C., and E. C. Apling, B.Sc., A.R.I.C.

This is a special post-graduate evening course of lectures and practical work based on the syllabus for the examination leading to the Diploma of the Royal Institute of Chemistry (Branch E). The course will extend over two complete sessions: 1st Year—Tuesdays and Thursdays, 6-9 p.m. (commencing September 26); 2nd Year—Mondays and Thursdays, 6-9 p.m. (commencing September 25).

The syllabus is as follows:

Food.—Origin, composition and methods of analysis. Identification and determination of adulterants, impurities, preservatives. Vitamins. Microscopical examination of food.

Drugs.—Chemistry, analysis and microscopy of drugs: their pharmacological action and therapeutic values. Alkaloidal assay and examination of pharmaceutical preparations. Chemotherapy.

Water.—Chemical and bacteriological examination of water, sewage and effluents. Microscopical examination of water deposits.

Bacteriology.—Milk, water, food preservation, food poisoning. Sterilization. Antiseptics and disinfectants.

Toxicological Analysis.—Detection of poisons and blood stains. Examination of urine.

Act and Regulations relating to Food, Drugs and Poisons.

Copper Roofing for New Research Laboratory

Ruberoid copper roofing, manufactured by The Ruberoid Co. Ltd., has been used to screen from electrical interference one of the new Pharmacological and Biochemical Laboratories at the Beecham Research Laboratories, Betchworth, Surrey. The laboratory is being used for electro-physiological experiments—such as measuring the potential in nerve fibres—which would be vitiated by outside interference.

For this purpose it was necessary to sheath all the internal surfaces, ceiling, walls and floor, with a good conductor in continuous electrical contact at earth potential, and to ensure that the multiple service pipes or conduit were isolated from outside interference. Ruberoid copper roofing was selected because it proved to be more pliable and there was a considerable saving in cost compared with plain copper sheeting. Fixed by the normal adhesive method, it was then anchored with nails to the timber battens set into the walls and ceiling. Copper strips were soldered after fixing over all the joints to achieve electrical continuity.

Copper faced internal shutters screen the window and door openings under actual test conditions. A plenum air conditioning system supplies and extracts fresh air through ceiling vents, which are screened with fine copper gauze.

Manufacturer's News

Griffin-Grundy Furniture Link

Griffin & George Ltd. and Grundy Equipment Ltd. have been associated for several years in the manufacture and marketing of metal laboratory furniture and laboratory apparatus, including service racks, Griffin-Grundy ovens, Grundy solenoid valve, etc.

A still closer link has now been forged whereby the Grundy Group becomes responsible also for the manufacture of wood laboratory furniture at their Cowley, Middlesex and Mitcham Joinery Works. Marketing will continue to be handled solely by Griffin & George (Laboratory Construction) Ltd., whose Sales Department is operating from High Street, Cowley, Uxbridge, Middlesex.

The new organization provides a unique comprehensive service comprising, in one location, both engineering and wood working facilities attuned to the specialized requirements of laboratory installations.

A new catalogue is now being compiled of laboratory furniture illustrating and describing the complete range of units and fittings in wood and metal.

Pakistan Distributors for Quickfit & Quartz

Quickfit & Quartz Ltd. have appointed Azam Trading Corporation of Karachi, as their exclusive distributors in both West Pakistan and East Pakistan.

Microwave Instruments Ltd.

The Directors of Hilger & Watts Limited announce that their company has acquired the whole of the issued share capital of Microwave Instruments Ltd.

Mr. J. Bilbrough, A.M.Brit.I.R.E., will continue to be managing director of microwave Instruments Ltd., whose factory is at North Shields in Northumberland. They are precision and electronic engineers, well known for their waveguide components and microwave test equipment, which are complementary to those for millimetre wavelengths manufactured by Hilger & Watts. The two companies have been collaborating for over a year in the development of measuring apparatus for electron spin resonance.

G. & G. Exhibitions

Two exhibitions are being staged by Griffin & George in Cardiff and Manchester at which modern laboratory equipment will be shown.

They will be held in the Welsh College of Advanced Technology, Cathays Park, Cardiff, from September 12 to September 14 inclusive and at the Chorlton Town Hall, Cavendish Street, Manchester, from September 18 to September 21 inclusive.

Q. & Q. Appoint Greek Distributors

Quickfit & Quartz Ltd., manufacturers of interchangeable laboratory glassware, have appointed P. Bacacos, Chemical and Pharmaceutical Products Co. Ltd., of Athens, as their exclusive distributors in Greece.

Southern Analytical in Moscow

Southern Analytical Ltd. participated in the exhibition section of the fifth International Congress of Biochemistry held in Moscow from August 10 to 16, and showed a wide range of polarographic equipment including the Southern-Harwell pulse polarograph and Davis differential cathode ray polarograph together with other instruments of interest to the analyst.

Mr. H. M. Davis, Director and Chief Engineer of Southern Analytical, was in attendance, together with Mr. C. Wontner and Mr. J. Southon.

U.K. Balances for International Bureau

Stanton Instruments Ltd. have recently supplied and installed a 10 kg. capacity high precision balance at the Bureau International des Poids et Mesures, Sèvres, near Paris.

The Bureau, which is the world's standards laboratory for those countries which adhere to the International Metric Convention, is re-equipping its Mass Section during the next few years. The 10 kg. balance is the first new high precision balance to be installed since 1910 and orders for further high precision balances have already been placed with Stanton Instruments Ltd., for delivery during the next two years.

The 10 kg. balance mentioned above is of the conventional three knife edge design and is fitted with a special optical reading system. It will be used for the testing of larger weights and is expected to provide a precision of weighing of not less than 1 in 100 million.

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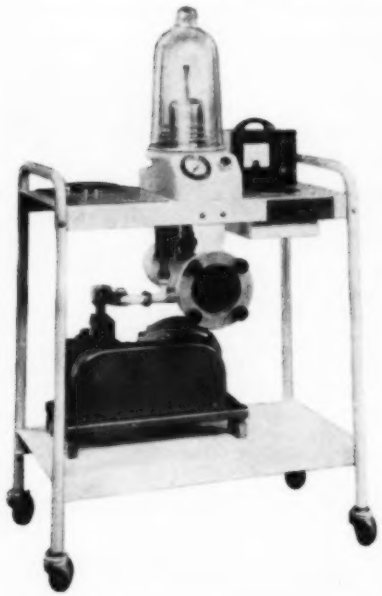

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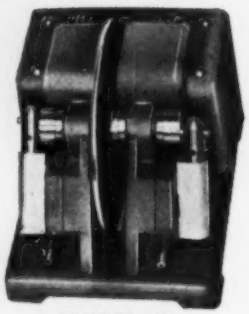

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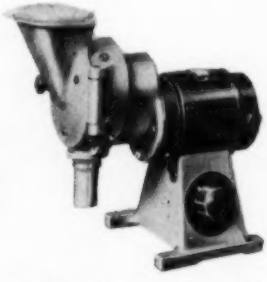

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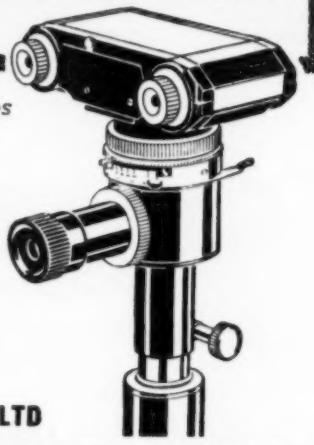

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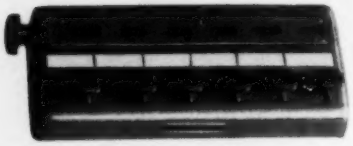

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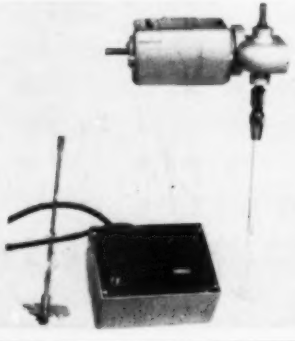

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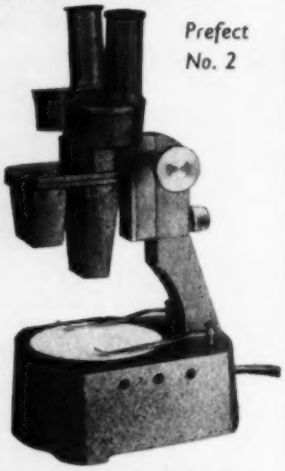

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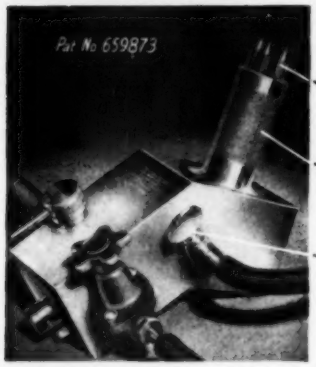

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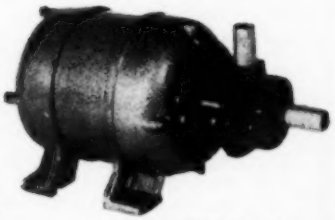

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South Liverpool Hospital Management Committee, Liverpool Chest Hospital, 68-70 Mount Pleasant, Liverpool, 3.

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Applications stating age, qualifications and full details of experience should be sent to the undersigned to be received not later than Monday, 18th September, 1961.

Garnet Chaplin,
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MEDICAL RESEARCH COUNCIL require a Senior Laboratory Technician or Laboratory Technician experienced in Bacteriology. Salary in accordance with age and experience, plus London weighting. Applications to The Director, Laboratory Animals Centre, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey. Telephone: Wallington 5512.

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Opportunities also arise in the laboratory for young men with G.C.E. 'A' level passes in Biology, Physics or Chemistry and enquiries are invited from such students.

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ARE You Interested In Animals Without being A Fanatic? Then write for free, illustrated prospectus to UFAW (The Universities Federation for Animal Welfare), 7A Lamb's Conduit Passage, London, W.C.1.

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No. 15

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(the above paper and *idem, ibid.* **29,** 1187).

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(K. Hayashi *et al., Talanta,* **4,** 126 (1960)) and for **phosphate** (*idem, ibid.,* **4,** 244 (1960)).

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